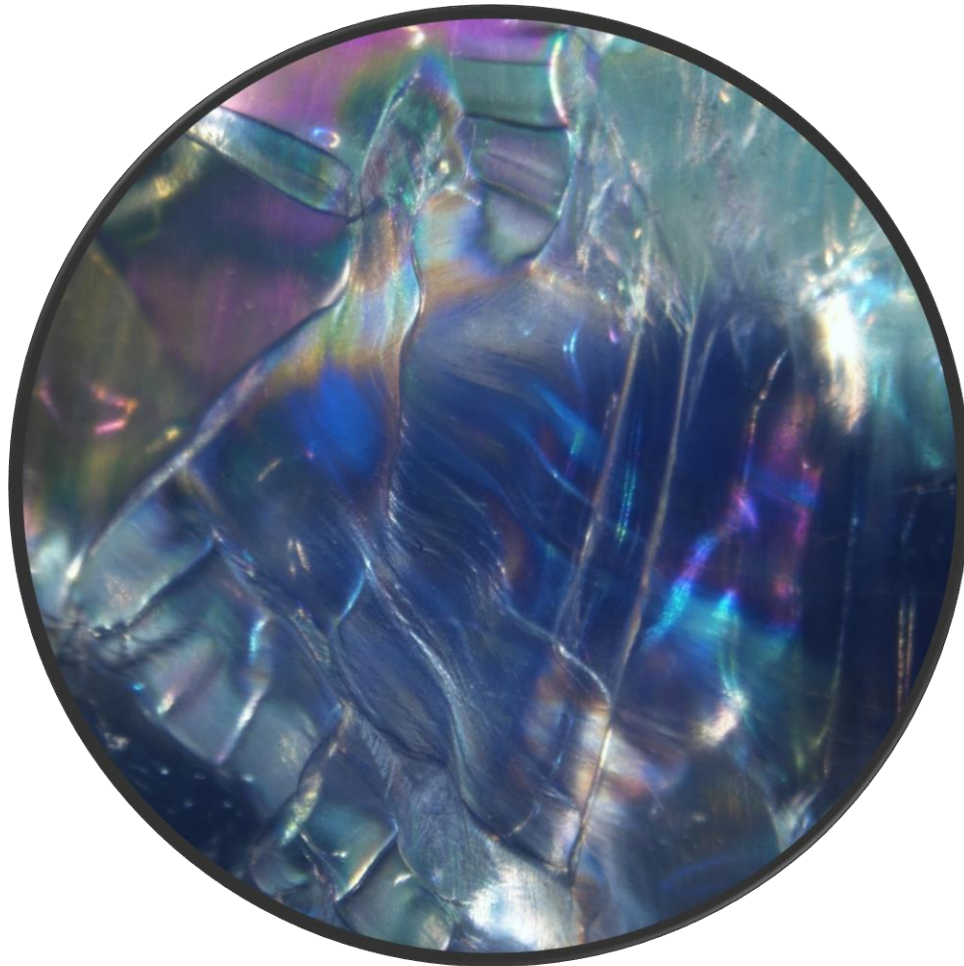


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# Polarized Light Microscopy



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## Contents

|   |    |
|---|----|
| Introduction .....                                    | 1  |
| Basics of Polarized Light .....                       | 2  |
| Polarized Light Microscope .....                      | 4  |
| Köhler Illumination and Microscope Setup .....        | 6  |
| Sample Preparation .....                              | 8  |
| Tools .....   | 8  |
| No Polars .....                                       | 10 |
| Single Polars .....                                   | 12 |
| Pleochroism .....                                     | 12 |
| Becke Line – Refractive Index Determination .....     | 12 |
| Dispersion Staining .....                             | 14 |
| Crossed Polars .....                                  | 17 |
| Isotropy/Anisotropy .....                             | 17 |
| Use of Waveplates .....                               | 18 |
| Birefringence .....                                   | 19 |
| Sign of Elongation .....                              | 21 |
| Conoscopical Images (Bertrand Lens) .....             | 22 |
| Overview and Additional Considerations .....          | 24 |
| Easily Identifiable Materials & Characteristics ..... | 24 |
| Identification Exercise .....                         | 27 |
| Identification Exercise Answers .....                 | 28 |
| References .....                                      | 29 |

## Introduction

Polarized light microscopy (PLM) is a technique which employs the use of polarizing filters to obtain substantial optical property information about the material which is being observed. This information can be combined with other microscopy techniques to confirm or elucidate the identity of an unknown material, determine whether a particular contaminant is present (as with asbestos analysis), or to provide important information that can be used to refine a manufacturing or chemical process. PLM was the major microscopy technique in use for identification of materials for nearly a century since its introduction in 1834 by William Fox Talbot [1], as other techniques such as SEM (Scanning Electron Microscopy), FTIR (Fourier Transform Infrared spectroscopy), XPD (X-ray Powder Diffraction), and TEM (Transmission Electron Microscopy) had not yet been developed. Today, it is still the only technique approved by the Environmental Protection Agency (EPA) for asbestos analysis, and is often the technique first applied for identification of unknown materials.

PLM uses different configurations in order to determine different material properties. With each configuration additional clues can be gathered, leading to a conclusion of material identity. With no polarizing filter, the microscope can be used just as a stereo optical microscope, and view qualities such as morphology, size, and number of phases. With a single polarizing filter (“single polars”), additional properties can be established, such as pleochroism, individual refractive indices, and dispersion staining. With two polarizing filters (“crossed polars”), even more can be deduced: isotropy vs. anisotropy, extinction angle, birefringence/degree of birefringence, sign of elongation, and anomalous polarization colors, among others. With the use of PLM many of these properties can be determined in a matter of seconds, even for those who are not highly trained. McCrone, a leader in the field of polarized light microscopy, often advised, “If you can’t determine a specific optical property of a particle after two minutes, move onto another configuration” [9]. Since optical properties can be seen so very quickly and easily under polarized light, it is only necessary to spend a maximum of two minutes on a technique to determine a particular property – though often only a few seconds are required.

## Basics of Polarized Light

Light normally vibrates in all orientations, but when light passes through a polarizing filter only light vibrating in a particular plane is allowed to pass through. This concept is utilized in sunglasses and in camera filters, where only vertically oriented light is allowed passage, completely blocking the horizontally oriented light that is the cause of glare (see Figure 1). This can be viewed in practice, as the rotation of the filter 90° to its normal orientation allows for the horizontally polarized glare to be allowed through and seen.



Figure 1. Effect of a polarizing filter. [3]

The concept of refractive index is also extremely important in PLM, and is essentially a measure of how fast light is able to pass through a material. As light passes from one material into another, if they have different refractive indices then the light will be bent (as seen in Figure 2). If the light is entering a more dense material, the bending occurs towards the normal, or towards the horizontal plane of the interface between the two materials. This is due to the fact that light actually moves slower in some materials as compared to others. This can be caused by a density difference (as in the case of water and air), or it can be due to crystal lattice orientations which may act to impede light waves. The definition of refractive index is as follows:

$$\text{Refractive Index (RI)} = \frac{\text{Speed of light through a vacuum}}{\text{Speed of light through a material}}$$

The result of this definition is that the lower the refractive index, the faster the light travels. For example, light travels almost as quickly through air as it does in a vacuum, with air having a refractive index of 1.003 [4]. Although it can vary rather significantly depending on the

composition, some types of glass can have a refractive index of 1.66. Determination of refractive index can aid in identification of the material, since every material's refractive index is different. Some materials have more than one refractive index due to material strain or crystal structures, and determination of these additional refractive indices can also be helpful in identification.

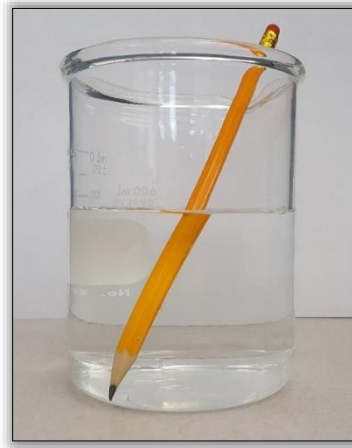


Figure 2. Light bending at air/water interface. [5]

## Polarized Light Microscope

In a transmitted light microscope, light enters from below the sample and passes through a polarizing filter, called a “polarizer,” before reaching the sample (see Figure 3, left). As mentioned previously, this polarizer restricts the exiting light to one plane of vibration. As it passes through the sample, this light is split into light vibrating at two orientations at right angles to each other, called the “ordinary” (o-ray) and “extraordinary” (e-ray) rays. Since these rays travel in different directions through a material, they can experience different effects and this can affect their individual speeds of travel through that material.

In materials that have only one refractive index (i.e., have the same optical properties in all directions), pass through the substance at the same rate and these two rays are in phase with each other. Once they reach the second polarizing filter (called the “analyzer”) these rays are recombined. Since they are *in phase*, they perfectly cancel each other out via destructive interference, and the sample can thus no longer be seen under cross polars. In materials that have more than one refractive indices (i.e., do not have the same optical properties in all directions), then the ordinary and the extraordinary rays become *out of phase* once they exit the material. When they recombine they result in “interference colors” that can be very beautiful – and of course provide additional clues for identification.

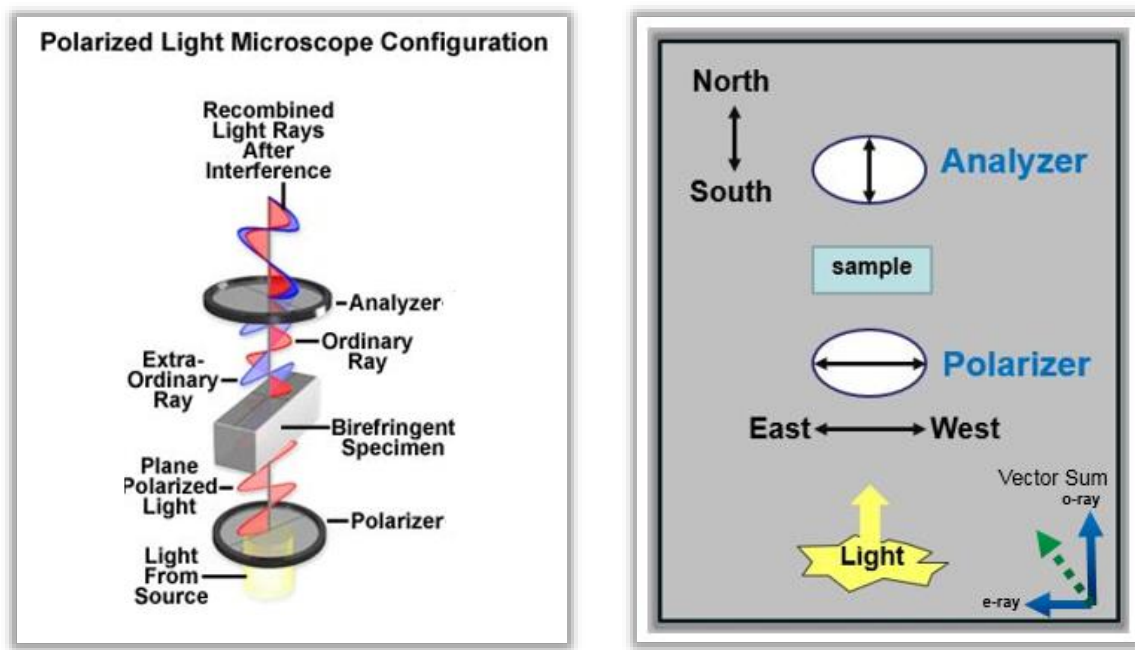


Figure 3. Polarizer configuration (left) and alignment (right). [6,5]

The polarizer and analyzer are set up at a  $90^\circ$  angle to each other (see Figure 3, right), so that the plane of light that the first filter lets through is blocked completely by the second filter. The polarizer is placed in “East-West” orientation, which only allows light vibrating in the East-West orientation to pass through. The analyzer is placed in the “North-South” orientation, which only allows light vibrating in the North-South orientation to pass through. The filters themselves are composed of polymer films embedded with iodoquinine sulfate crystallites oriented in the same direction (developed by Dr. Edwin Land in 1932) [7]. These crystallites absorb the ordinary ray and only allow the extraordinary ray to go through, thereby limiting the transmitted light to one plane. After the interaction of this light with the sample and upon passing through the analyzer, the combination of the o-ray and e-ray results in a vector sum of the two rays. When the sample is rotated to a  $45^\circ$  angle in relation to the polarizers, the sample will show a maximum brightness, but will show extinction (be completely dark) at  $90^\circ$  due to destructive interference of the light rays. The usual configuration of a PLM showing the placement of these parts can be seen in Figure 4.

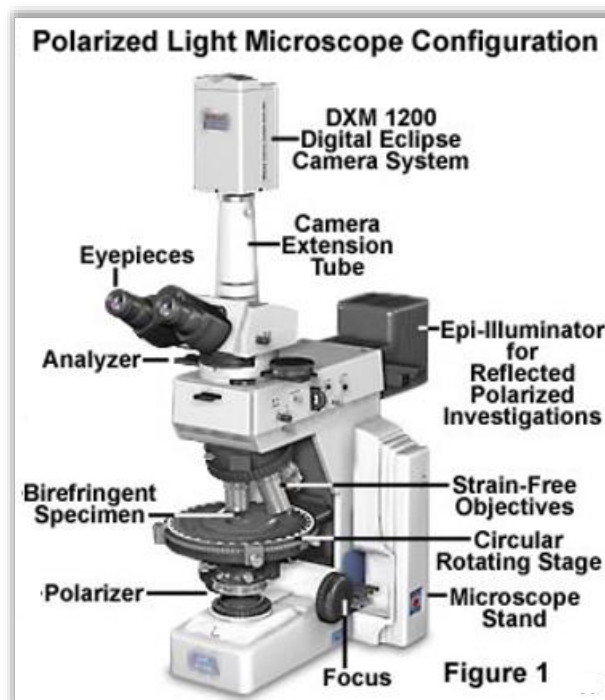


Figure 4. Polarized light microscope anatomy. [6]

## Köhler Illumination and Microscope Setup

Proper lighting is extremely important when using a microscope of any type, for it determines what features can be seen. Using the technique seen in Figure 5 to achieve Köhler illumination is one of the best ways of ensuring that a sample is well-lit. Essentially, the image of the light filament (the microscope's light source) is focused on the "back focal plane." This is the 2-dimensional plane that sits directly above the objectives (rather than below the objectives where samples are placed). As the light travels down to the specimen plane the image of the filament naturally defocuses. At the level of the sample it is impossible to see the filament, yet all of the light is allowed to reach the sample. This allows for full illumination.

The typical light source for a microscope is a tungsten filament, which glows when an electric current is passed through it. Depending on the setup, it may be possible to use knobs on the lamp housing to adjust internal mirrors which focus the filament so the individual coils can be seen in the light exiting the lamp housing. Once this is accomplished, a sample can be placed on the stage and the course and fine focus knobs can be used to bring the sample into focus. The field diaphragm can then be closed so that the edges of the light can be seen, and the image of the field diaphragm (an octagon-like shape) is focused by raising or lowering the substage condenser. The field diaphragm can then be centered using the substage condenser knobs. In systems with a diffuser, this is the final step before the field diaphragm is opened.

Technically, if a diffuser is present then "true" Köhler illumination is not possible, as the diffuser blurs the filament image. However, it is still possible to achieve very good illumination with the below method. In systems where no diffuser is present, it is necessary to add a step where either a Bertrand lens is inserted into the light path or the ocular (eyepiece) is temporarily removed in order to observe the back focal plane. The filament should fill the view and should be in focus. If not, then the lamp housing knobs can again be adjusted.

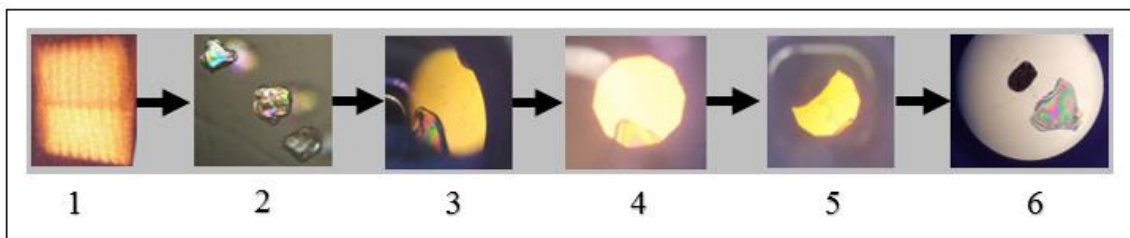


Figure 5. Köhler Illumination Steps. (1) Focus the light filament. (2) Bring the specimen into proper focus. (3) Partly close the field diaphragm so the edge of the light can be seen. (4) Focus the field diaphragm by raising/lowering the



substage condenser. (5) Center the field diaphragm by using the substage condenser knobs. (6) Open the field diaphragm slightly larger than the field of view. [All: 5].

This process *must be repeated whenever objectives are switched*, as otherwise the light (with the field diaphragm partially closed) will appear slightly off-center or with fuzzy edges, indicating that the illumination is not optimized. Although at first introduction following this procedure can appear somewhat complex, in practice it only takes seconds and can easily become second nature even after just a few repetitions. It is also necessary to insure that a sample stays centered in the ocular crosshairs upon rotation of the stage or change of objective. The method for this varies depending on the type of microscope, with some objectives “pre-centered” and others requiring a few very simple adjustments that are articulated in the instructions that come with the new objectives.

Although appropriate lighting of the sample is extremely important, it is also very important to have the microscope camera settings correct so that any images taken reflect true conditions. The white balance must be correct, or it will completely throw off the colors seen in the pictures – and when looking from the eyepieces to the image it will be obvious that they do not match. This can be calibrated by bringing part of the slide that does not contain the sample into view and using the auto-white balance function on the software (if it has one, which most do). If this does not correct the white balance then a standard white such as a Kimwipe or the back of a very white business card can be used to try and force it into the correct color scheme. Once the image matches what is seen in the live microscope, it is correct. It is highly preferable to have the correct colors showing when the picture is taken rather than during post-processing. This is because when the photo is taken it can be compared real-time against what is viewed through the eyepieces (rather than from memory, which can be fallible), and it helps with ensuring fidelity of the taken pictures.

It is important to note that transmitted light PLMs’ objectives are designed for use with coverslips, while reflectance PLMs have objectives that are designed for use without a coverslip. Although at times some information can be obtained, if the wrong setup is used then it will be difficult or impossible to obtain a completely clear image with magnification greater than 20 X. Transmitted light objectives are typically designed for use with particles or fibers that are at least

partially transparent, while reflectance objectives are more often used for mineralogical examinations.

### Sample Preparation

In order to properly view a sample, it is necessary to immerse it in an oil of known refractive index. This allows for a clearer view, as the refractive index of the oil more closely matches the refractive index of the coverslip and slide. It also allows for comparisons between the refractive index of the sample and that of the oil it is immersed in. This is done by placing a sample onto a glass slide and covering it with a coverslip, as seen in Figure 6. A drop of refractive index oil is placed on the glass slide at the edge of the coverslip. (This must be done without touching the dropper itself to the slide, or the oil bottle could be contaminated.) The oil wicks under the coverslip and can be viewed through the microscope as it moves.

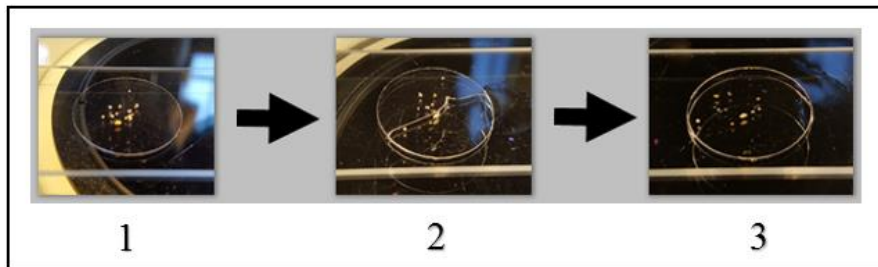


Figure 6. Sample Preparation Steps. (1) Place the sample on a clean slide and then place a coverslip on top. (2) Apply refractive index oil drop next to coverslip and allow it to wick across. (3) Continue until the sample is covered. [All: 5]

Depending on the size of the particles/fibers several drops may be required to completely fill the coverslip, though samples with thinner diameters may only require one drop of oil. Subsequent drops may hover momentarily on the previous oil film prior to becoming part of it, which can cause the oil to splatter or move as it does so. Therefore proper positioning is important and may take some practice in order to optimize placement. After this is complete, excess oil may be wicked away with a Kimwipe, taking care not to accidentally introduce oil onto the top of the coverslip. If oil is introduced to the top of the coverslip it is possible to contaminate the objectives with oil, which obscures the image and causes unnecessary cleanup where there is potential to scratch the objectives' lens.

### Tools

Since small fibers and particles are frequently handled during PLM analysis, it is extremely important to have the correct tools for the manipulation of samples. Needles are

attached to probe holders for gripping during particle handling. The needle should be appropriately sized to the size of particles or it will be exceedingly difficult to handle the particles and to not lose them. Tweezers are often used as well for particles or fibers. Glass slides are used to mount the samples, but often come with particles or smudges despite being “pre-cleaned.” Therefore they must be washed with soap and water, rinsed with DI water, and dried with a Kimwipe. The coverslips do not typically come as dirty, however they should still be checked and if the analysis could be influenced by this kind of contaminants then they should also be washed. Additional care should be taken during the washing of coverslips as they are much thinner and easier to break than glass slides, especially during drying.

## No Polars

The term “no polars” implies that there is no polarizing filter in place on the microscope – i.e., this microscope mode views materials as would a “normal” (stereo) optical microscope. The main optical properties are those which simple magnification and good lighting can convey. These include properties such as morphology, size, and number of phases. Morphology is simply all the details which contribute to how something “looks,” and a surprising amount of information about the identity of a particle or fiber can be obtained from this step alone. Similarly to how one recognizes a person (hair length, glasses/no glasses, clothing style, facial structure), particles exhibit characteristics which can be used to identify them. Color, surface features (smoothness/roughness, fracture pattern, general shape, fiber twist), and transparency/opacity all contribute to how a particle or fiber looks. Many of these features can be seen in Figure 7, which shows solids found in filtered lube oil from Crawler #2. Analysts must learn particles one at a time, collecting a mental database of what particles look like. Though this skill is invaluable, it is not trivially mastered and can take years and much dedication to develop.

Size can also provide important information, as some fibers have a larger diameter than others due to the manufacturing (or natural) process that created them. For particles, size contributes to the ability of the particles to settle from liquid suspensions (such as in paints), or to even fit comfortably under a coverslip for additional observations. A last but significant quality that can be determined under no polars is the number of phases present – i.e. how many different materials appear to be present. If the material does not appear homogenous, then a few particles from each different material can be removed and analyzed in further detail.

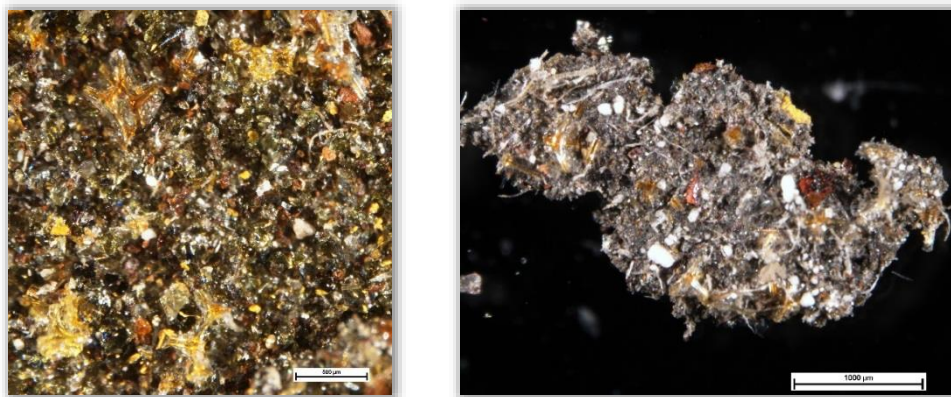


Figure 7. Crawler solids under no polars (left – high magnification, right – low magnification). [8,9]

An additional consideration concerns the concept of transparency. Very thin materials can appear transparent when viewed alone, but in aggregation can exhibit a definite color. A good example of this would be paraffin wax. The crystals that make up this wax are themselves transparent, but in aggregation they result in the scattering of light and a resultant color. At first introduction this information may seem like an additional complication, but simply noting that a material exhibits a different color when the particles are together as compared to when they are apart can provide an additional clue to the material's identity.

## Single Polars

Under normal optical microscopy, the average refractive index is what is seen. The use of a single polarizing filter allows for determination of *individual* refractive indices. This is of course easiest if the material has only one refractive index, but can also be done for materials with more than one by rotation of the stage to view the desired refractive index.

### Pleochroism

Materials with more than one refractive index may exhibit a phenomenon called pleochroism. Instead of seeing just the average color of a material, under single polars the color the material appears changes depending on the orientation of light. This requires either rotation of the sample or for the sample to have several particles/fibers oriented in different directions. In Figure 8 crocidolite asbestos fibers are shown. Vertically oriented fibers show grey colorization, while horizontally oriented fibers show blue coloring.

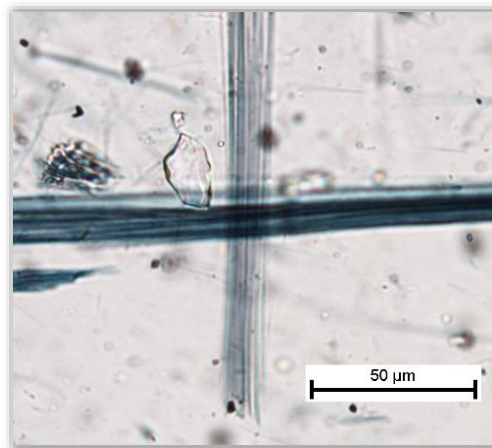


Figure 8. Example of Pleochroism – Crocidolite asbestos, gray (vertical) and blue (horizontal). [8]

### Becke Line – Refractive Index Determination

Determination of a material's refractive index can be very helpful for identification. Various materials can often have very different refractive index values, and taken with other information (morphology, crystallinity, etc.) the refractive index can be a major clue as to the identity of the material.

To do this, the sample is first immersed in an oil of known refractive index, and then a monochromatic filter is placed in the light path. The *distance* between the sample and the

objectives is *increased*, and the white line of light that surrounds the particle either moves inward or outward from the particle. This line, (seen moving inward in Figure 9) is called the Becke line and is the result of light being diffracted around the particle. Which direction it goes is dependent on whether the particle or the oil has the higher refractive index – the white line goes into the material with the higher refractive index.

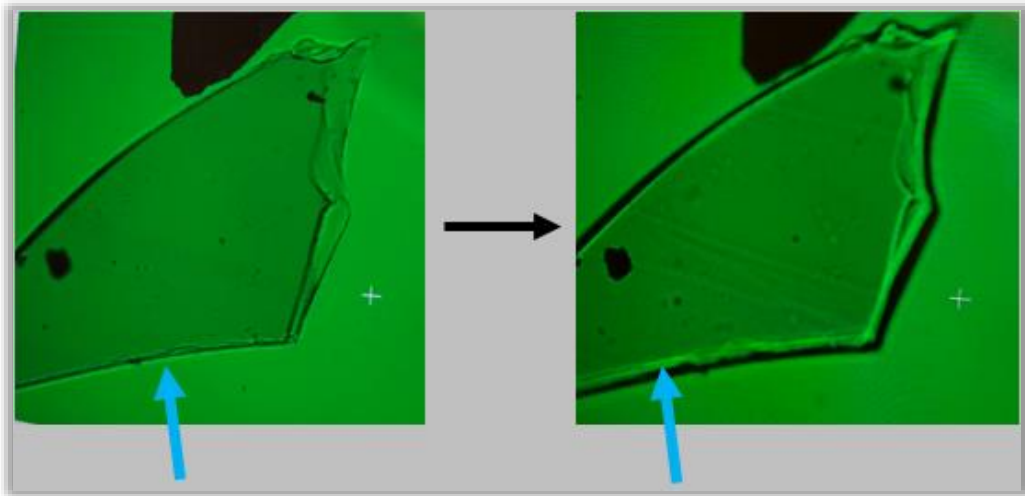


Figure 9. Becke line for isotropic glass. [8,8]

Once this is known, oils with different refractive indices can be used to iteratively determine the refractive index of the particle. When the refractive indices match, two Becke lines form (one orange and one blue). If the particle is just slightly out of focus then the orange line will be lining the internal edge of the particle, and the blue line will be lining the outside of the particle. In this way, the refractive index of a particle or fiber can be narrowed down to as precise as  $\pm 0.002$  refractive index units.



Figure 10. Filament triacetate in 1.55 refractive index mineral oil. [8]

Figure 10 above shows a close refractive index match between a filament triacetate fiber and the oil it is immersed in (under single polars). Notice that the shadowing of the edge of the fiber is very faint, and it is somewhat difficult to clearly discern where the fiber stops and the oil starts. On an interesting side note, filament triacetate is simply cellulose that has been treated with acetic acid or a similar solution in order to substitute acetyl groups for its hydroxide groups. This allows it to be more stable in organic solvents and thus allows it to be spun into a long fiber as the solvent evaporates [10].

### Dispersion Staining

Although “staining” usually implies some kind of dye is involved, dispersion staining instead uses light to non-permanently color a material. It is used most often especially for the identification of asbestos as required by the EPA (ASBESTOS (bulk) by PLM NIOSH Method 9002 [12]), such as seen in Figure 11. First, a “central stop” is placed in the center of the light path, forcing light to diffract around the material. (Note that an annular stop can be used, but it results in a light rather than a dark background and therefore makes it more difficult to see the colorations.) This results in a colorized outline of the particles or fibers.

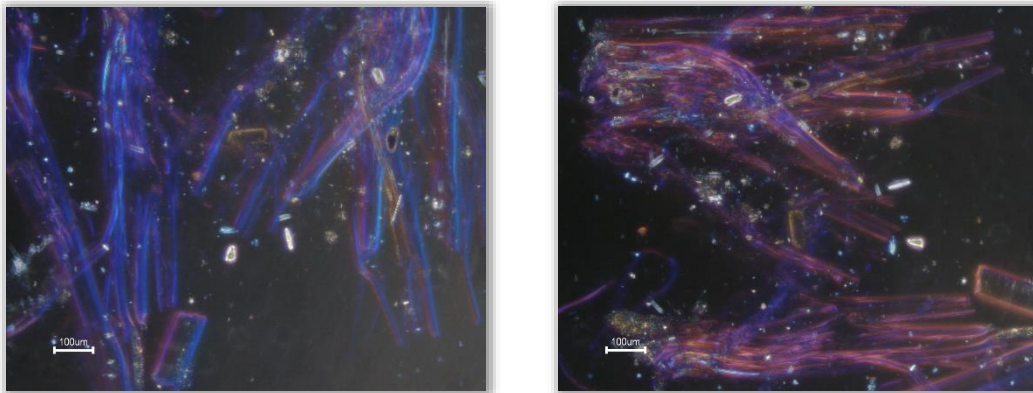


Figure 11. Dispersion stained chrysotile asbestos (Left – parallel, blue. Right – perpendicular, magenta.). [9,9]

A nice aspect of this technique is that one can actually choose which color to look for by choosing the wavelength of light that is being used (with blue as a common color of choice). Once this choice is made, then the sample must be mounted in an oil that matches its refractive index at that wavelength (see Figure 12). This requires knowledge of the material’s exact refractive index. This can be determined by the Becke line test, or if the material identity is known then its refractive index should be able to be found in a reference manual. Once mounted,



the sample can be checked – if the particle or fiber that is being looked for is there, it should show the expected color. Samples that do not show that particular color are of some other material.

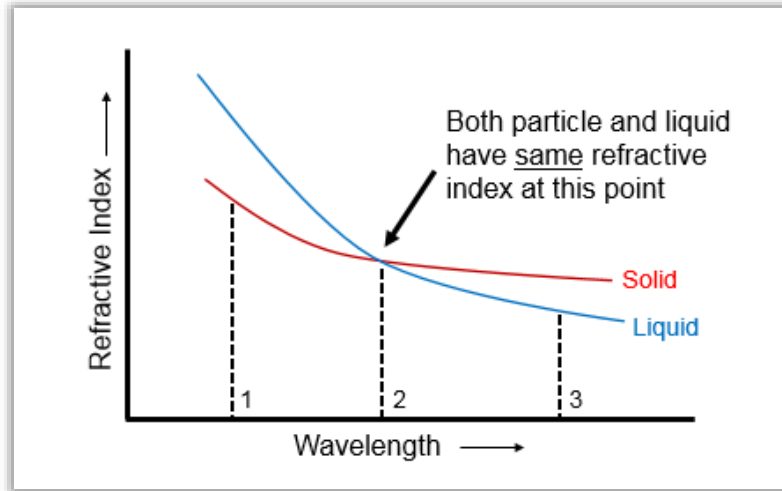


Figure 12. Dispersion staining curve. [5]

Dispersion staining is typically used to determine if a particular material is present (as with asbestos), though it can also be used to judge the homogeneity of a material such as in Figures 13 and 14. In Figure 13, there is only one material present since every particle has a light blue outline of exactly the same shade. In Figure 14 there are only four particles that are of the same material (the purple-pink outline), and everything else is a dissimilar material.

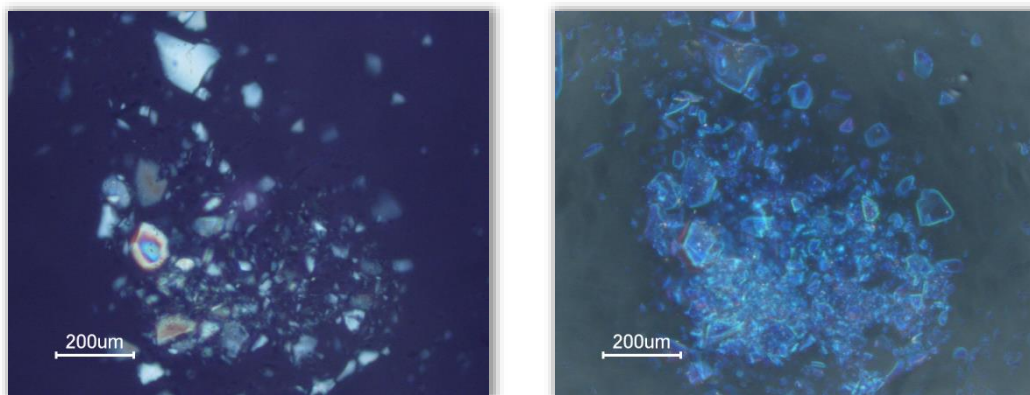


Figure 13. Quartz under crossed polars (left) and under dispersion staining (right). [9,9]

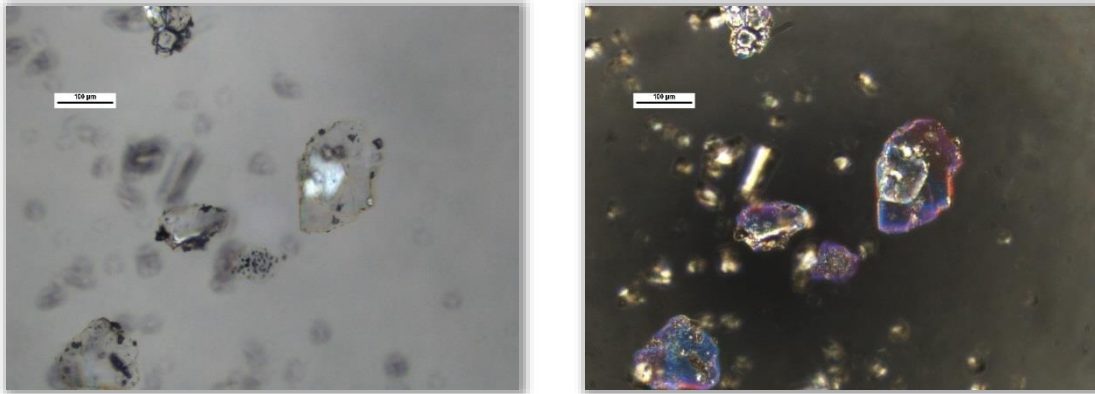


Figure 14. Non-homogenous material under single polars (left) and dispersion staining (right). [9,9]

One complication is that many materials have more than one refractive index. Therefore they may show different colors under dispersion staining for different orientations, as seen in Figure 11. These are predictable, however, as chrysotile asbestos will always show blue in parallel orientation and magenta-purple on perpendicular orientation. Other materials may show blue-blue, which would mean that material is not asbestos (or at least not this type). Another issue is that some material's second refractive index will not intersect with the immersion oil in the visible range of wavelengths, in which case the sample will show white or yellow upon being placed in the orientation associated with that refractive index. If that occurs a different oil must be used for the second (or third) refractive index.

## Crossed Polars

There are several major advantages to using crossed polars. Under this mode it is very easy to determine whether materials are crystalline. Since most materials in existence do have some sort of crystalline structure, this can be very helpful as then additional properties can also be found. If the thickness is known then the birefringence (the difference between two refractive indices) can be estimated and vice versa. The directions along a material which light travels faster or slower can be found (sign of elongation). The crystal type (uniaxial vs. biaxial) can also be determined, which allows for the narrowing down of possible crystal lattice configurations.

### Isotropy/Anisotropy

The term “isotropic” refers to materials have a single refractive index, while “anisotropic” materials have at least 2 and sometimes 3 different refractive indices. Under single polars these refractive indices can be seen as different colors depending on the crystal’s orientation with respect to the polarizer (pleochroism). Under crossed polars, anisotropic materials show distinct bands of color (such as in Figure 15, right), while isotropic materials do not show up at all. Both isotropic and anisotropic materials can be seen under crossed polars if there is a  $15^\circ$  offset, as seen in Figure 15 (left). Crystals are typically anisotropic, though a few crystal structures are isotropic. Sodium chloride (NaCl) has a face-centered cubic structure (FCC) type of crystal lattice that is the same in all directions thus only one refractive index (i.e., isotropic).

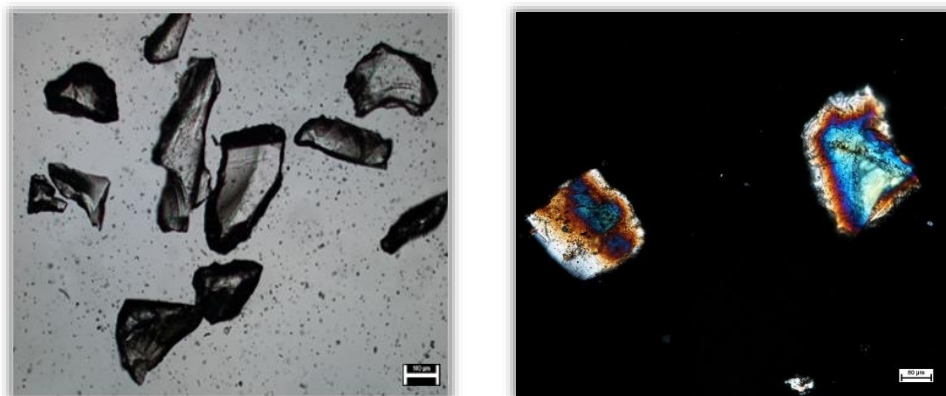


Figure 15. Isotropic glass (left), anisotropic quartz (right). [8,8]

### Use of Waveplates

Waveplates are sometimes also called “retardation plates” and they add or subtract a specified number of wavelengths to what is being viewed. As light travels through an anisotropic material, one of the rays will be slower than the other (i.e. “retarded”). Wavelength is added/subtracted depending on if the slow vibration direction of the material is aligned with the slow or fast vibration direction of the waveplate. If aligned addition will occur, if not subtraction.

One of the most common is the lambda  $\lambda$  (red) waveplate seen in Figure 16b that adds 546 nm of wavelength. This allows for more separation between color bands (enhancement of optical path differences) and thus easier counting. The quarter-wave plate (seen in Figure 16c) adds about 125 nm and converts the linearly polarized light to either elliptical or circularly polarized light, depending on the orientation of the polarizers. This is typically used for viewing anisotropic materials whose refractive indices are very similar, though it is also used in other applications to eliminate undesired reflections.

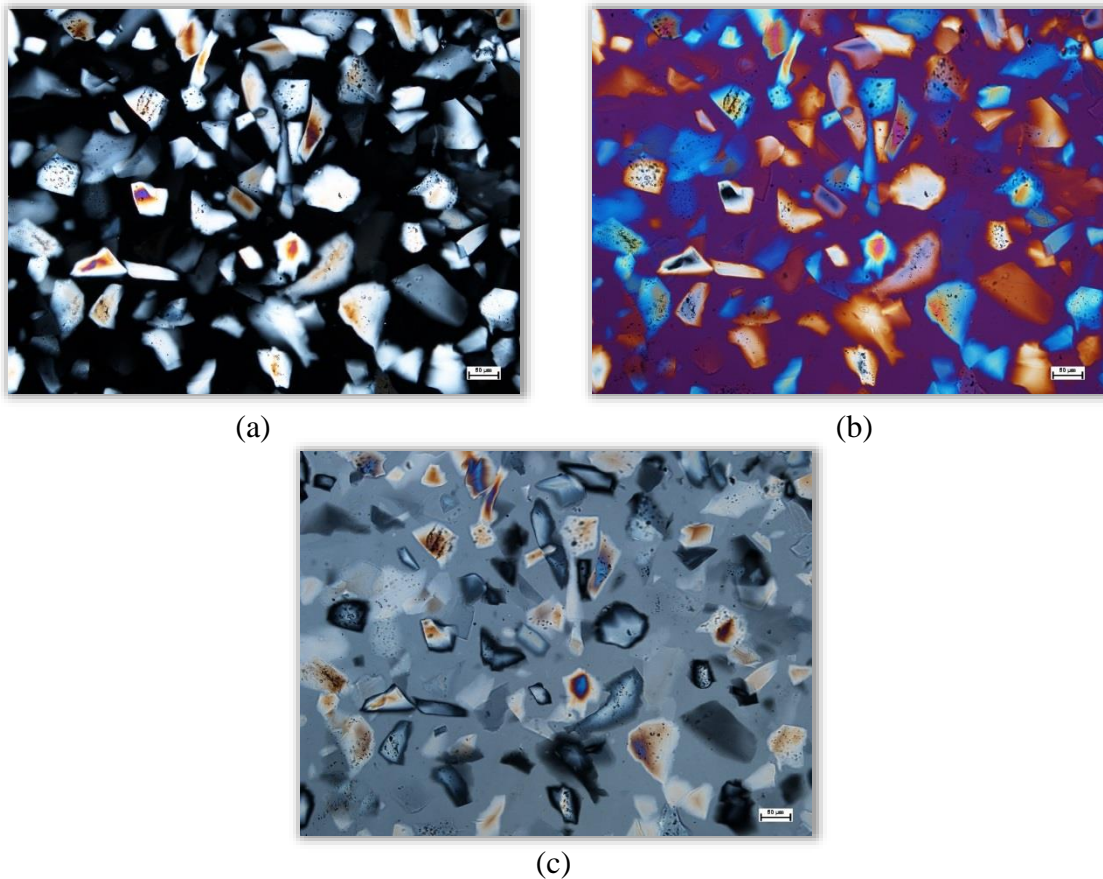


Figure 16. (a) CP with no waveplate. (b) CP with red waveplate. (c) CP with quarter-wave plate. [8,8,8]

## Birefringence

Birefringence results from the ordinary and extraordinary rays becoming out of phase in anisotropic materials and causes the material to exhibit very nice rainbow-like colors (see Figure 17) when these rays are recombined at the analyzer. Unlike pleochroism, these “interference colors” can be seen without rotation of the specimen. Only *crystalline* materials (which include most polymers) and *strained amorphous materials* exhibit birefringence (such as the strained plastic bag in the cover photo [8]). Crystalline materials include only materials which have a particular crystal lattice formation at the molecular level, though up to 85% of all materials do have this property [2]. Amorphous materials do not exhibit this structure as molecules are oriented more randomly. However, upon experiencing strain they may align and thereby exhibit birefringence.

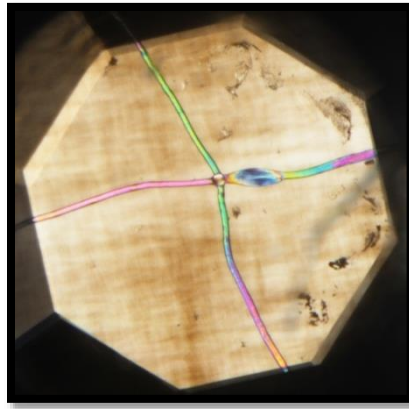


Figure 17. Polyester fiber showing birefringence. [8]

There is a relationship between the degree of birefringence, the optical path difference, and the thickness of a material. The amount that the two rays are out of phase is the birefringence, or  $n_1 - n_2$ , and can be assigned a numerical value. Birefringence is a direct result of the difference between the principal refractive indices. The “optical path difference” is the difference in distance that each ray had to travel, and is sometimes also referred to as the “retardation,” and the interference colors are the visible effect of this optical path difference. In isotropic materials finding the numerical value for this is simple and can be done by multiplying the specimen thickness by the materials’ single refractive index. In anisotropic materials, the numerical value for birefringence is substituted for the refractive index value, for example:

$$\text{Optical Path Difference} = (n_1 - n_2) * t \text{ (thickness)}$$

This relationship is more clearly shown on the Michel-Lévy chart, shown in Figure 18. Interference colors can be first order, second order, and so on. Color bands progress initially from shades of gray and white (since this is in the UV range, the color is “washed out”) to yellow, orange, pink, red, purple, blue, green, back to yellow, and so on, similar to the color order of a rainbow. At one point of each order is a red, and this is typically what is counted as it is one of the easiest colors to see (i.e. “first order red,” “second order red,” etc.). Very thick materials such as the quartz in Figure 19 can be counted up to a point, but the thickness (or birefringence) can be so large that the individual interference colors can no longer be differentiated. Red waveplates can be used to add or subtract wavelengths, but if that is not enough the material may need to be ground to a smaller particle diameter. The first order contains greys and whites and signifies a very low birefringent material, though high order birefringent materials can also show nearly white coloring. This can make it difficult to differentiate them, however under a red waveplate they can usually be distinguished.

If the interference color and thickness is known, then the numerical value of birefringence can be determined, shown with blue arrows in Figure 18. The highest interference color is found on the bottom x-axis and followed up to the y-axis value associated with the known thickness. The line which intersects this is then followed to the value of birefringence. (Note that the possible values of birefringence stretch from the top x-axis all the way to the right hand side of the graph.) Similarly, if the birefringence and interference colors are already known, the thickness can be determined.

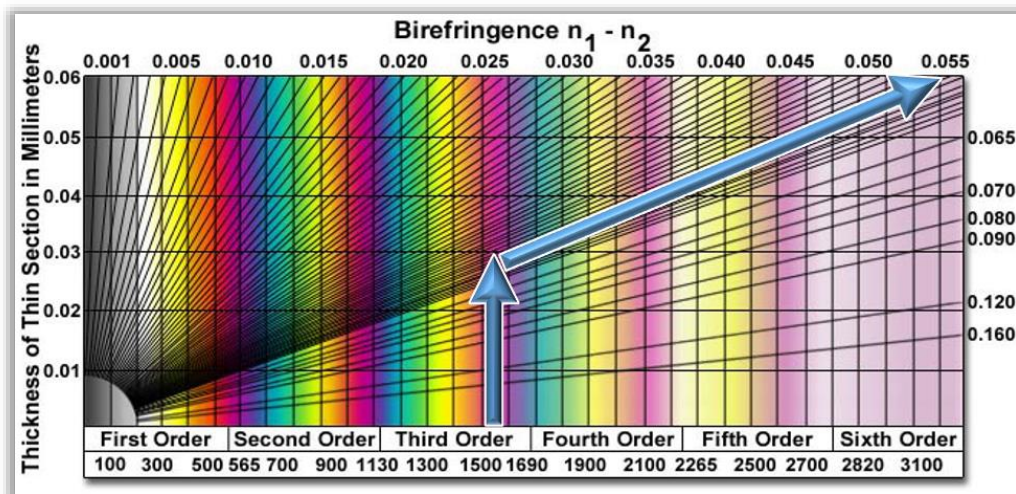


Figure 18. Michel-Lévy Birefringence Chart. [11]

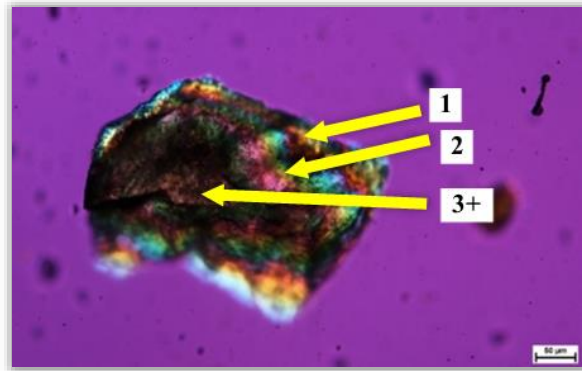


Figure 19. Quartz under crossed polars and red waveplate. [8]

### Sign of Elongation

The sign of elongation refers to the direction in which light travels fastest within a material. Fibers often have a *positive* sign of elongation, which means that light travels faster when going across a fiber (perpendicular  $\perp$ ) and slower when going along the fiber (parallel  $\parallel$ ). This results in the fiber showing blue when oriented SW to NE, and yellow when oriented NW to SE (under cross polars and red waveplate). An example of this can be seen in Figure 20. A *negative* sign of elongation would show the opposite colors, i.e. yellow when oriented SW to NE, and blue when oriented NW to SE (as seen in Figure 21). Although nearly all fibers have a positive sign of elongation, there are some notable examples including acrylic, cotton, polyester, nylon, and even some biological materials such as collagen. Crystals are more varied, with some types having a positive and some a negative sign of elongation (such as crocidolite asbestos).

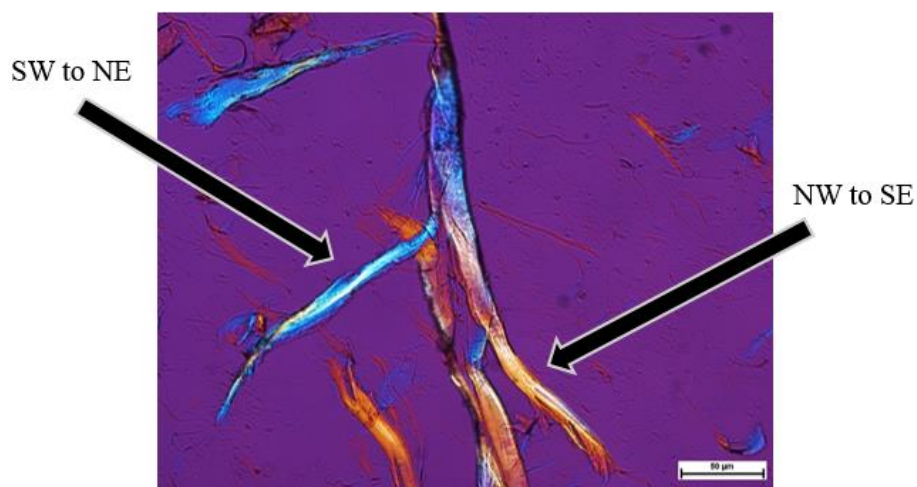


Figure 20. Wood pulp showing positive sign of elongation. [8]

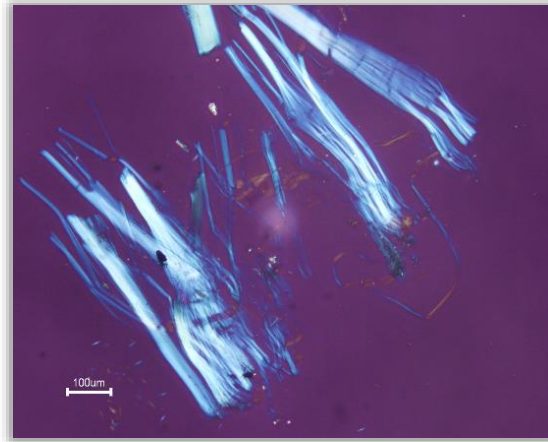


Figure 21. Crocidolite asbestos fibers showing negative sign of elongation. [9]

### Conoscopical Images (Bertrand Lens)

With the use of a Bertrand lens, it is possible to view the back focal plane and see “conoscopical interference figures.” In this case, it is necessary to have a diffuser in place so the image of the lamp filament does not overlay the image. The use of a Bertrand lens can be helpful in verifying the crystallinity of a particle, as crystalline particles will show a very specific set of possible images, whilst non-crystalline particles will not show an image at all (and will simply be grey across the entire view). Interference figures are black and white and the darker bands show areas of extinction, though Figure 22 shows the figures with a red waveplate added in order to determine sign of elongation. It is important to have the crystal of interest centered in the viewpoint (using a 40X objective) without additional materials nearby, as they can affect the light and result in a useless image.

The orientation of the crystal influences the interference figure that appears. For crystals viewed by looking down on the top of the crystal, (the crystal is essentially “sitting up”), the result is shown in Figure 22. Uniaxial crystals have a “Maltese cross” or hourglass shape, while biaxial crystals look almost as though they have two eyes. When positioned this way, the center of the uniaxial figure is the optic axis. For biaxial crystals, the center of each eye is an optic axis. Interference figures for different crystal positions can be seen in reference books or other similar materials. Uniaxial crystals are associated with a certain set of crystal structures (tetragonal, hexagonal, rhombohedral), as are biaxial crystals (orthorhombic, monoclinic, triclinic). One complication with orientation is that in certain orientations uniaxial and biaxial crystals may not show interference figures. The crystals must then be rolled to a better orientation to view.



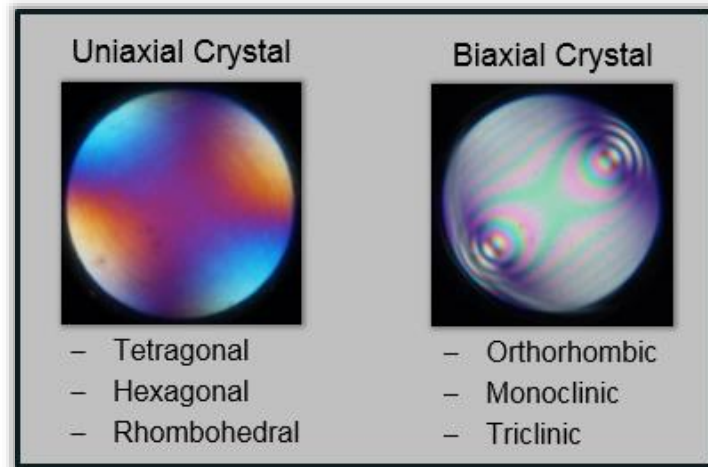


Figure 22. Interference figures of uniaxial and biaxial crystals from a polymer film. [9,9]

Although the determination of a crystal as uniaxial vs. biaxial is helpful, two further pieces of information can be obtained with the addition of a red waveplate. Recall that crystals are typically anisotropic. *Uniaxial* crystals have *two* refractive indices, with one of them aligned to the optic axis and *biaxial* crystals have *three* refractive indices. The optic sign of a crystal is related to the relative magnitudes of the crystals' refractive indices. The optic sign (which can be positive or negative) is found by looking at whether the red waveplate results in addition (blue) or subtraction (yellow) in a particular quadrant. For example, the uniaxial crystal in the figure is uniaxial (-) since the lower left (SW) and upper right (NE) quadrants are yellow, and the opposite quadrant set is blue. If a crystal was uniaxial (+) then these would be switched. For biaxial crystals a similar operation can be done, however the crystal must be turned to an orientation 45 degrees from extinction rather than looking directly down the optic axis as is necessary for uniaxial crystals. For biaxial crystals only, optic angle (also called 2V angle) is found from determining the acute angle of the arcs' curvature, and can be used to determine the actual crystal structure.

## Overview and Additional Considerations

A summary of the properties available to view in the three different possible PLM configurations is seen in Table 1, noting that determination of optical axial angle and optic sign are more advanced techniques.

Table 1. PLM abilities for each configuration.

| No Polars  | Single Polars   | Crossed Polars  |
|--|---|---|
| <ul style="list-style-type: none"> <li>• Shape/Morphology</li> <li>• Size</li> <li>• Color</li> <li>• Surface features</li> <li>• Transparency/Opacity</li> <li>• Number of phases</li> <li>• Homogeneity</li> <li>• Aggregation</li> <li>• Presence of associated substances</li> </ul> | <ul style="list-style-type: none"> <li>• Pleochroism</li> <li>• Individual refractive indices                             <ul style="list-style-type: none"> <li>▪ Becke line</li> </ul> </li> <li>• Dispersion staining</li> </ul> | <ul style="list-style-type: none"> <li>• Isotropy vs. Anisotropy</li> <li>• Extinction</li> <li>• Retardation</li> <li>• Birefringence</li> <li>• Degree of Birefringence</li> <li>• Sign of Elongation</li> <li>• Anomalous polarization colors</li> </ul> |
|  |   | <ul style="list-style-type: none"> <li>• Optical axial angle</li> <li>• Optic sign</li> </ul>   |

### Easily Identifiable Materials & Characteristics

There are some additional morphology characteristics that are easy to recognize when viewing materials. Delustering is a process wherein titanium dioxide (TiO<sub>2</sub> – also called “rutile”) is added to a synthetic fibers to make them less bright and usable in clothing. These particles can be added in low, medium, or heavy concentrations in order to control the luster. The dark speckling of the acrylic fiber in Figure 23 (left) is the rutile particles embedded within the fiber. The way a material fractures can also aid in identification. Conchoidal fractures, which appear as a certain type of scalloped edge, can be seen on the left side of the top particle of glass in Figure 23 (middle-left). Cotton fibers (Figure 23 middle-right and right) display a certain kind of twisting. Mercerized cotton (i.e. cotton that has been through chemical treatment to increase its luster) still displays some of this twisting but not to as great a degree as do natural cotton fibers.

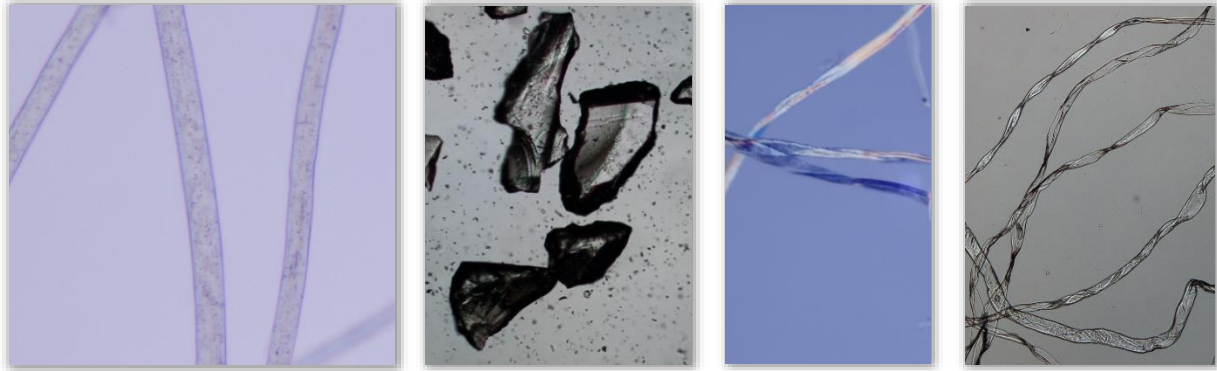


Figure 23. Acrylic fiber (left), Glass (middle-left), and Cotton (middle-right and right). [8,8,8,8]

Paper fibers (not shown) are flatter and broader than cotton, and do not show cotton's characteristic twisting. Fiberglass (silicon dioxide,  $\text{SiO}_2$ ) is anisotropic and has very thin fibers. Rockwool is also made of silicon dioxide and has long thin anisotropic fibers, similar to fiberglass. However, the manufacturing process for Rockwool is much messier, and results in a much less uniform distribution of fiber diameters as well as odd-looking tadpole-like bits of material, as seen in Figure 24. Rockwool and fiberglass are both used for thermal insulation, though Rockwool is typically found in thick mats and fiberglass is more often found woven or sprayed into a moderately thin fabric. Fiberglass can also be combined with epoxy to create fairly strong molds.

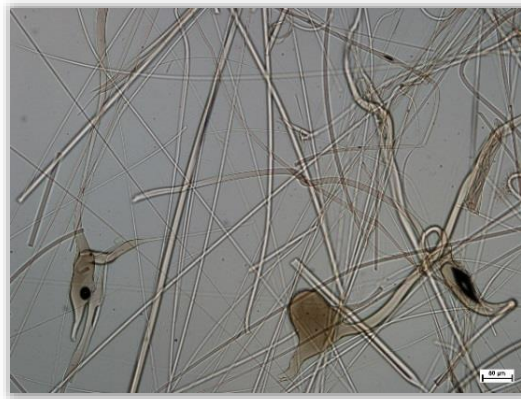


Figure 24. Rockwool. [8]

Occasionally biological materials such as diatoms (a type of algae), radiolarians (a type of protozoa), or foraminifera (a type of protest) can find their way into materials (as seen in Figure 25). It is helpful to be able to recognize them, as they may indicate non-sterile water intrusion.

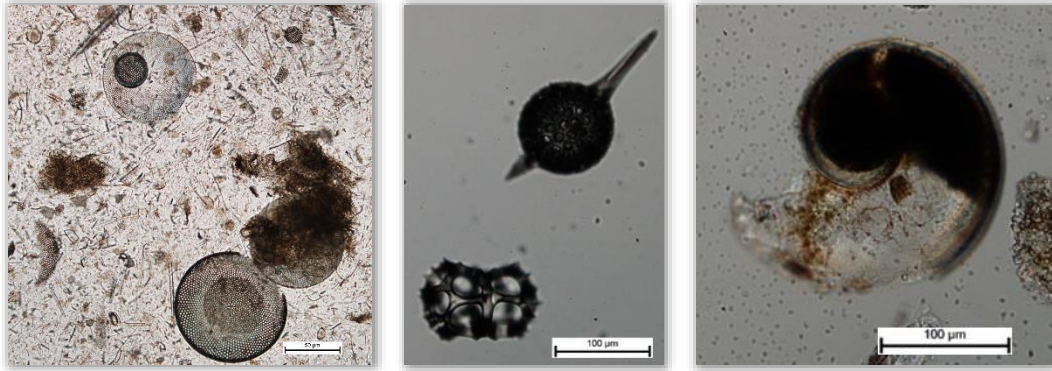


Figure 25. Diatoms (left), radiolarians (middle), and foraminifera (right). [8,8,8]

Other biological materials that may be found during examination of materials include pollen, such as those shown in Figure 26, or the trichomes as seen in Figure 27. Trichomes are parts of the underneath of plant leaves and their shapes differ by plant type, lending to their use in identification.

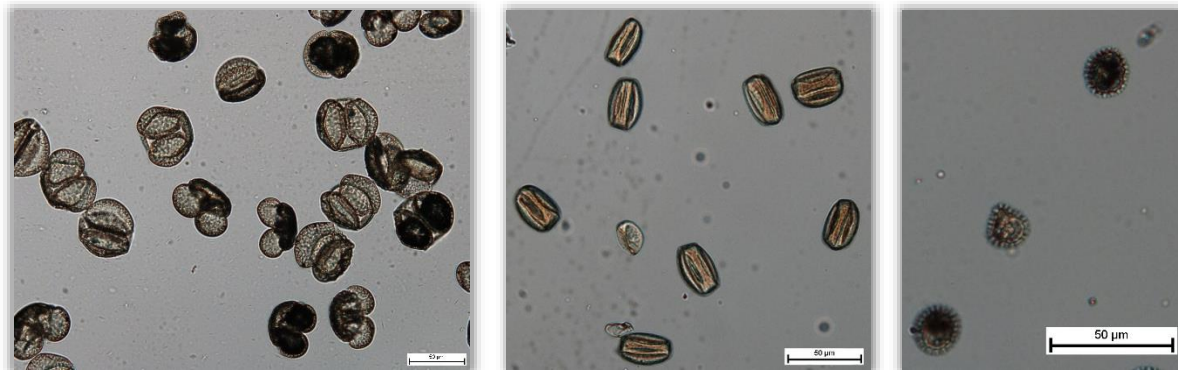


Figure 26. Pine pollen (left), red oak pollen (middle), and ragweed pollen (right). [8,8,8]



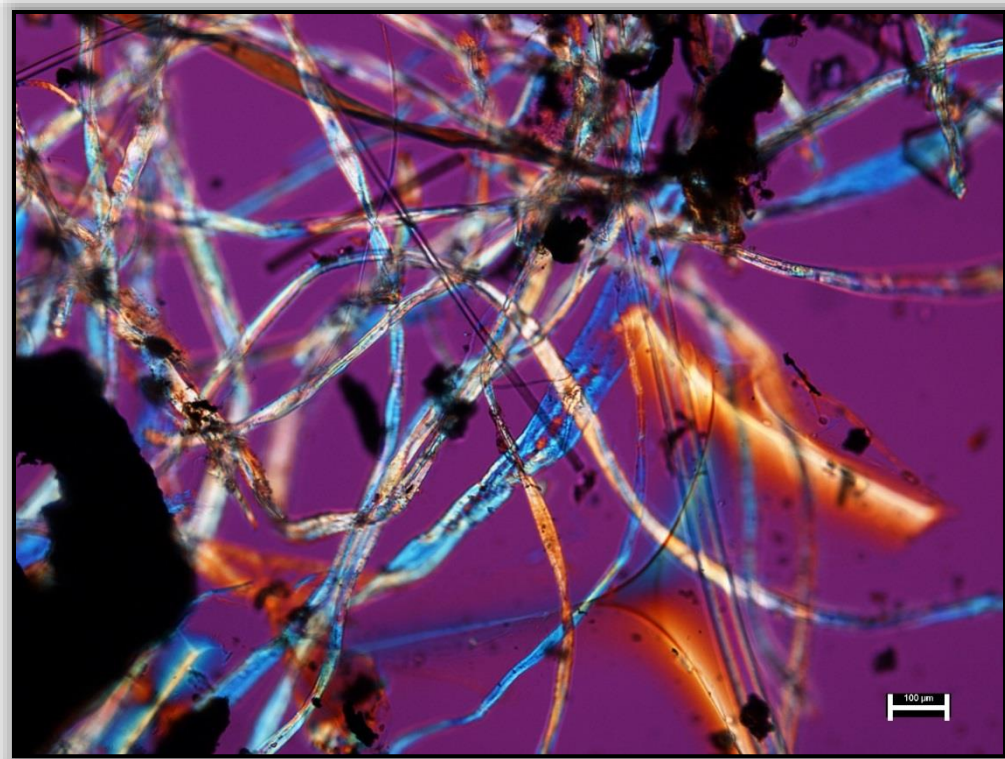
Figure 27. Sycamore trichomes. [8]

### Identification Exercise

Skills are most effectively integrated when practiced.

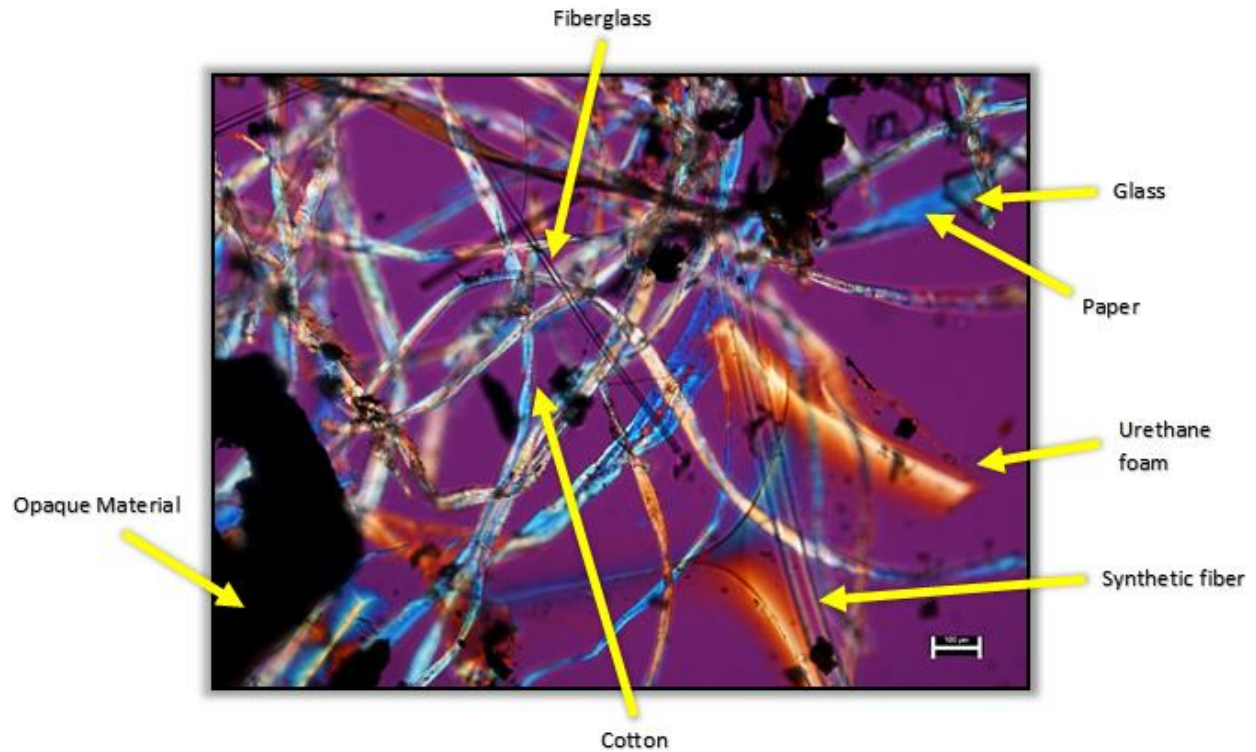
The below image is of various materials which were removed from a gear box lube oil filter on one of the trucks of Crawler #2 [9].

1. How many different materials (i.e., phases) can you see?
2. Are there any specific characteristics that you recognize?
3. What set-up of the PLM was used for this image?



Identification Exercise Answers

1. 7 different materials (i.e., phases) are shown:



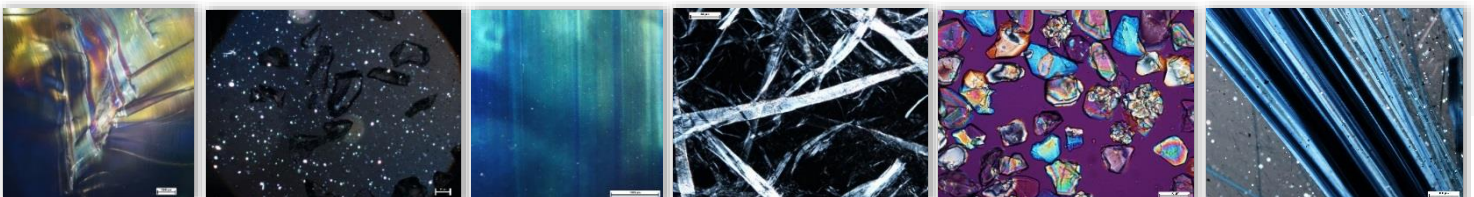
2. All of the materials have a different morphology.

- a. The cotton, paper, and urethane foam all have a positive sign of elongation.
- b. Fiberglass – long and straight, 10 micrometer diameter, isotropic fibers.
- c. Synthetic fibers – high birefringence, 25 micrometers, round fibers
- d. Cotton fiber characteristic twisting, 15 micrometers
- e. Paper – flat and broad, 75 micrometers
- f. Glass – isotropic particle
- g. Urethane foam
- h. Opaque material

3. Crossed Polars with a Red Waveplate (CP, RWP).

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All:  
[8]