

**SELECTED TOPICS FROM
ESSENTIALS
OF
POLARIZED LIGHT MICROSCOPY**

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Essentials of Polarized Light Microscopy, Fifth Edition (January 2008)

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Cover Photomicrograph:

Ammonium nitrate, NaNO_3 , recrystallized from the melt; crossed polarizers, 100X

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1. INTRODUCTION

Particles are minute fragments of, usually, solid or semisolid matter. They may be of a nuisance variety (e.g., household dust, air pollution particulate matter, incinerator-stack fallout), a health hazard (e.g., asbestos fibers, mold spores, hay fever pollen, silica dust [see Figure 1-1]), an industrial contaminant (e.g., dirt in photographic film, automobile paint, integrated circuits, magnetic tape, paper products, etc.), a pharmaceutical contaminant (e.g., glass flakes, silicone rubber, drug degradation product, thermally degraded paper fiber), or any desirable product, which just happens to be at or just below the threshold of unaided human vision. Today, the threat of terrorist attacks involving *Anthrax* and other bioterrorist agents—and the hoaxes, which inevitably accompany or follow true attacks—make the identification of “White Powders” imperative. In every case, the outstanding characteristic of all types of particles is their small size, so the use of magnifiers or microscopes is the obvious approach to studying and identifying tiny particles. This approach is hundreds of years old—as old as microscopes themselves. Particles have been characterized by polarized light microscopy long before modern instrumental methods, such as X-ray diffraction. Serious systematic particle characterization dates to the confluence of crystallography and the introduction of Nicol prisms to the microscope in the early part of the nineteenth century.

The modern particle characterization laboratory still has, as its principal investigative instrument, the polarized light microscope, or polarizing microscope, as it is sometimes called, along with its companion

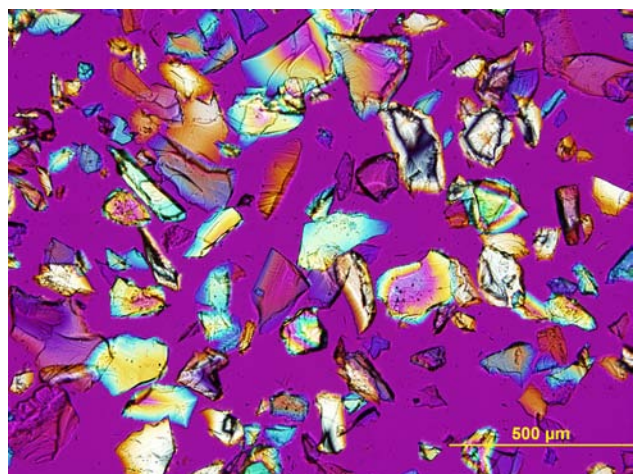


Figure 1-1. Quartz, crossed polarizers, first-order red compensator, 100X

low-power stereomicroscope. The addition of X-ray diffraction, scanning electron microscopes (SEM) and transmission electron microscopes (TEM), especially in their more recent X-ray analytical configurations, electron and ion microprobes, micro-Raman, micro-Fourier transform infrared (FTIR) spectrometer, and the many new kinds of microscopes currently under development have made the microscopist’s job of identifying particles easier, faster, and more comprehensive. The fact remains that in a laboratory where all of these analytical instruments are available the first instrument used on any particle problem is the low-power stereomicroscope, followed by the polarized light microscope. More than 95% of all particles can be characterized by the experienced microscopist with these two instruments alone, and until about 40 years ago that is about all there was available that could be used. In the past, if a tiny opaque particle was recovered, a portion of it was put into solution either through direct dissolution, or fusion followed by dissolution, and then individual cations, anions, and functional groups were tested for using the methods of microchemistry. Today, with the aid of an ion microprobe, not only can the elements present be determined, but because of the instrument’s incorporated mass spectrometer, even the isotopes of the elements present can be ascertained. Of course instruments like this cost in excess of one-half million to one million dollars. Most particle-characterization laboratories cannot afford expensive analytical instruments of this kind, so they start with low-power stereomicroscopes and polarized light microscopes, and then add perhaps a scanning electron microscope with X-ray capability, and a Fourier transform infrared spectrometer. With these, the vast majority of particles can be characterized, and the remaining can be characterized by laboratories that specialize in this area.

It is proposed here to describe the kinds of low-power stereomicroscope and polarized light microscope that are needed to characterize and identify particles, together with their setup, alignment, illumination, and methods of use. Ancillary equipment, accessories, chemical reagents, and techniques are also discussed.

To understand the equipment needed, it is first necessary to understand the characteristics of particles that are to be observed.

2. IDENTIFYING CHARACTERISTICS OF PARTICLES

There is quite a large number of identifying characteristics of particles that can be seen or ascertained with the aid of a polarized light microscope, the most important of which include:

1. Morphology: The shape of a particle; is it equant (about the same dimension in all directions), see Figure 2-1, acicular (needlelike), see Figure 2-2, or does it resemble a rod, plate, tablet, flake, cylinder, or sphere? For synthetic fibers, morphology refers to the cross-sectional shape.

2. Size: What are the particle's linear dimensions and thickness?

3. Surface texture: Is it smooth? porous? rough? scaly? Are there surface spines, ridges, furrows, sculpturing (sculpting) or markings of any kind (see Figure 2-3)? Are there tool marks evident?

4. Hardness: Does it deform or "squosh" when depressed and stay that way, or does it recover its shape (elastomeric)? Is it brittle? Does it break into a few or "zillions" of pieces? Does it seem hard and impossible to break?

5. Reflectivity: Is it very dull, semi dull, or highly reflective? In other words, how "bright" is the sample?

6. Transparency: Is it transparent, translucent, opaque, or some combination (see figure 2-4)?

7. Color: Is it colorless or colored by transmitted

light (see Figure 2-5)? What color is it by transmitted and/or reflected light?

8. Magnetism: Is the particle magnetic?

9. Refractive index/indices: This is perhaps the single most important identifying characteristic of transparent particles! The refractive index is the ratio of the velocity of light through a particle relative to the velocity of light in a vacuum. The internal, atomic structure of the particle may result in one, two, or three principal refractive indices. See Figure 2-6, in which the calcite particles have two refractive indices, indicated by the two markedly different contrasts in immersion medium $n_D=1.662$.

10. Pleochroism: A change in color or hue in a colored, transparent particle that has more than one refractive index, i.e. anisotropic, when its position is changed (e.g., stage rotated) relative to the vibration direction of the polarizer.

11. Dispersion staining: An optical method of imparting color to colorless, transparent particles; related to the refractive index relationship between particle and mounting medium, so that more than one color may be observed. See Figure 2-7 for particles with two or three principal refractive indices. The color(s) in a particular refractive index medium is/are characteristic.

12. Birefringence: The numerical difference between the principal (high and low) refractive indices. This may be determined through a measurement of

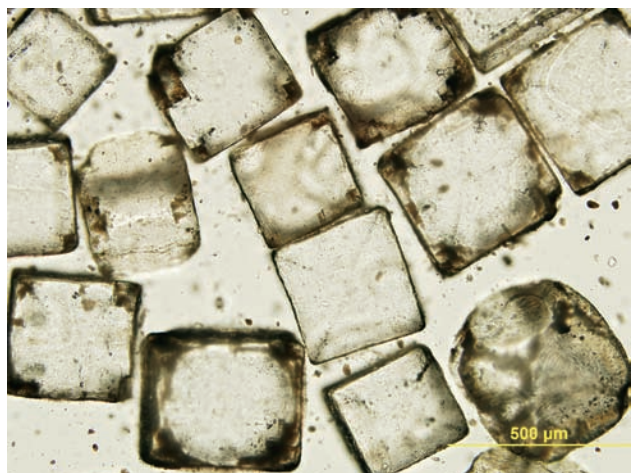


Figure 2-1. Table Salt, plane-polarized light, 100X

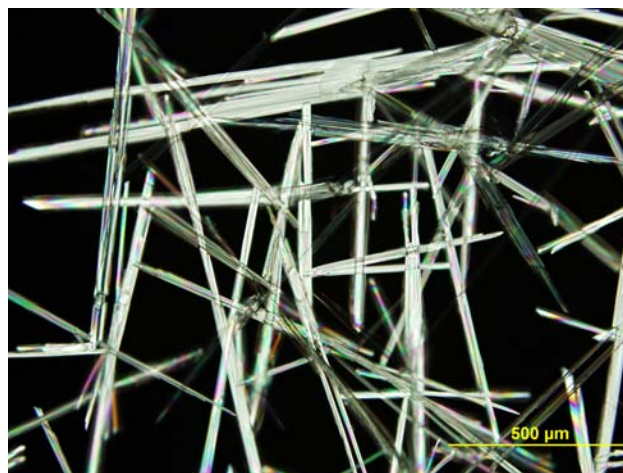


Figure 2-2. Salicylic Acid, crossed polarizers, 100X

the refractive indices, or through observation and interpretation of the dispersion staining colors, but can be more quickly and efficiently done qualitatively through observation of the retardation (interference color) in conjunction with the particle's thickness.

13. Extinction angle: The angle between the particle's extinction position and some prominent crystal face, length, or cleavage plane.

14. Sign of elongation: In particles which are elongated, the location of the high and low refractive indices relative to the elongated direction (see Figure 2-9).

15. Interference figure: An optical phenomenon observed at the back focal plane of a high-power (i.e., high numerical aperture) objective when viewing anisotropic crystals or minerals between crossed polarizers, with the Bertrand lens engaged. Its appearance is related to particle's orientation and birefringence (see Figure 2-10).

16. Melting point: Does the particle melt when heated? At what temperature? Does the particle sublime, decompose, or explode? Is there evidence of a polymorph (different forms for the same composition), or eutectic (an alloy or solution having the lowest melting point possible), or addition compound, or solid solution? (sublimation, see Figure 2-11; polymorph, see Figure 2-12)

17. Chemical composition: What elements, ions, functional groups make up the particle? See Figure 2-13 for an example of a microchemical determination of potassium.

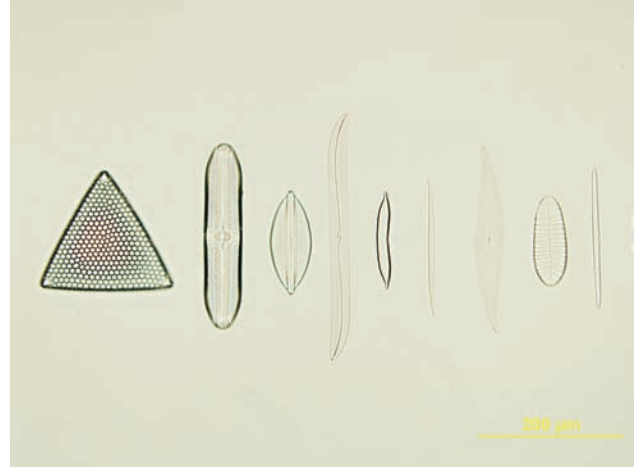


Figure 2-3. Arranged diatoms, plane-polarized light, 100X

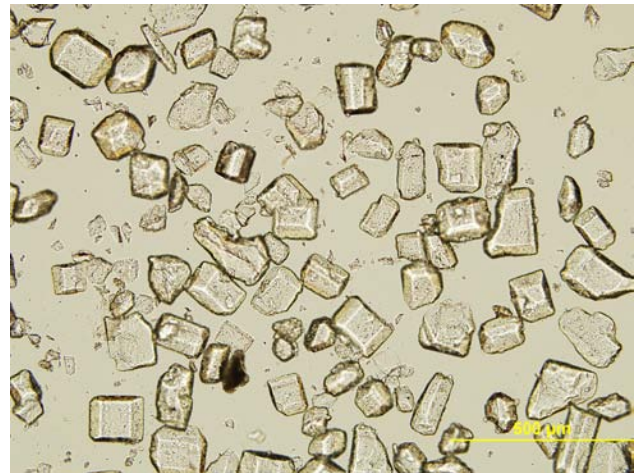


Figure 2-4. Sucrose, plane-polarized light, 100X

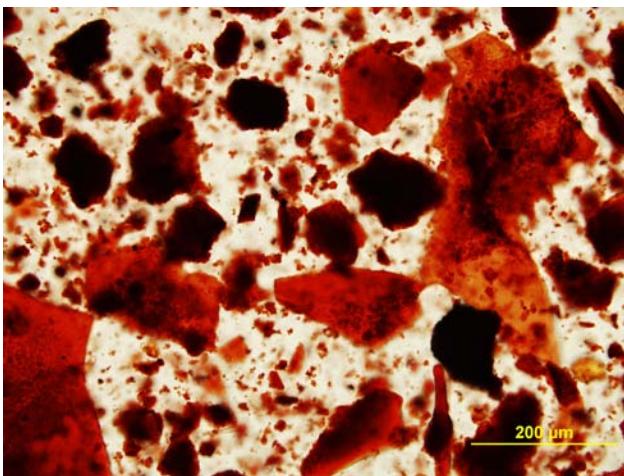


Figure 2-5. Cochineal, plane-polarized light, 200X

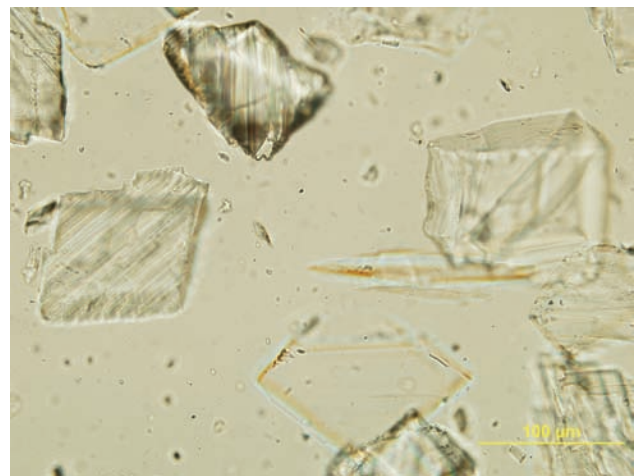


Figure 2-6. Calcite, plane-polarized light, 400X

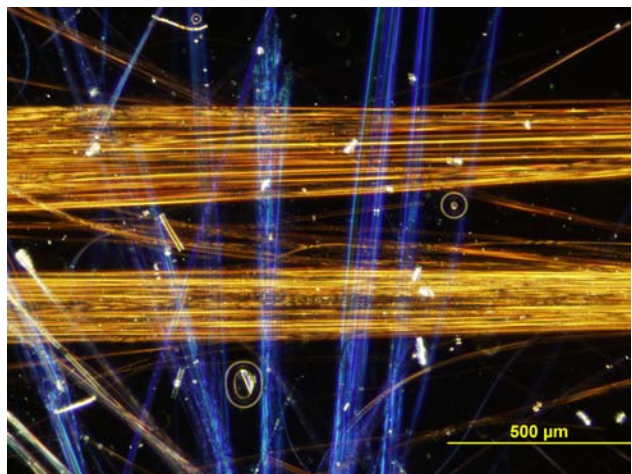


Figure 2-7. Amosite, central stop dispersion staining, 100X

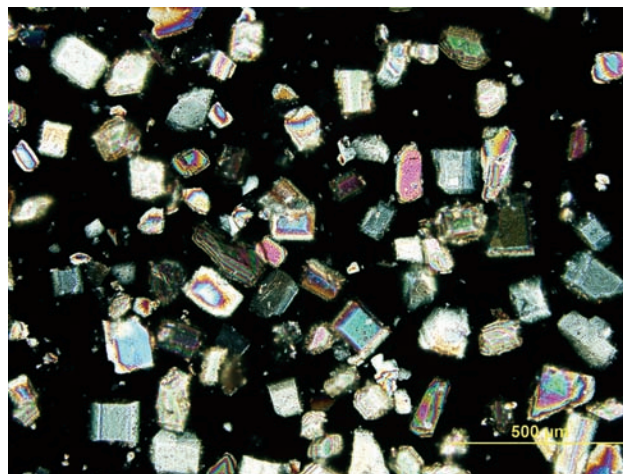


Figure 2-8. Sucrose, crossed polarizers, 100X

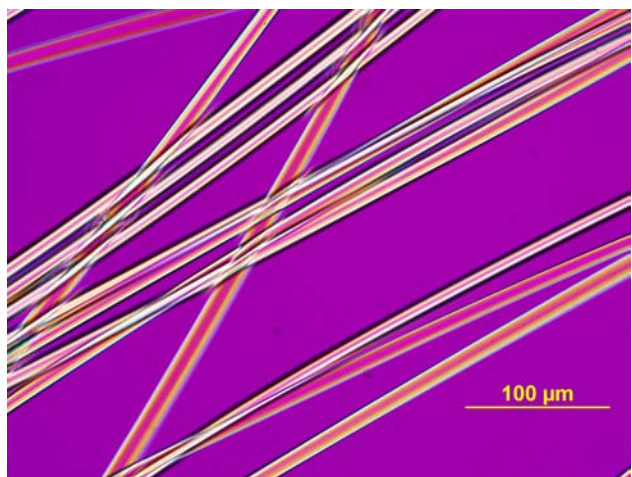


Figure 2-9. Rayon, crossed polarizers, first-order red compensator, 400X

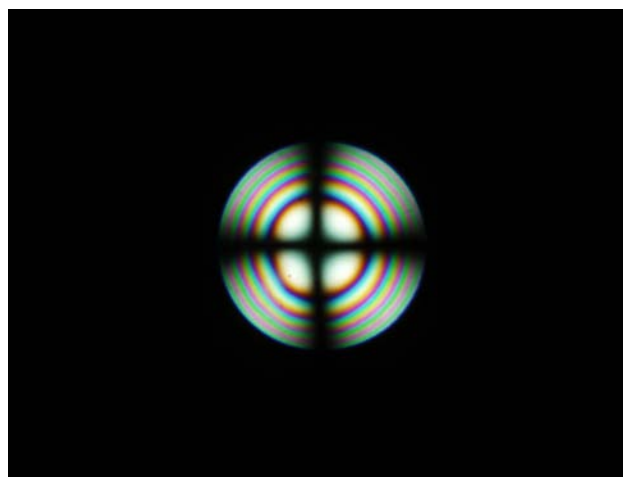


Figure 2-10. Uniaxial interference figure

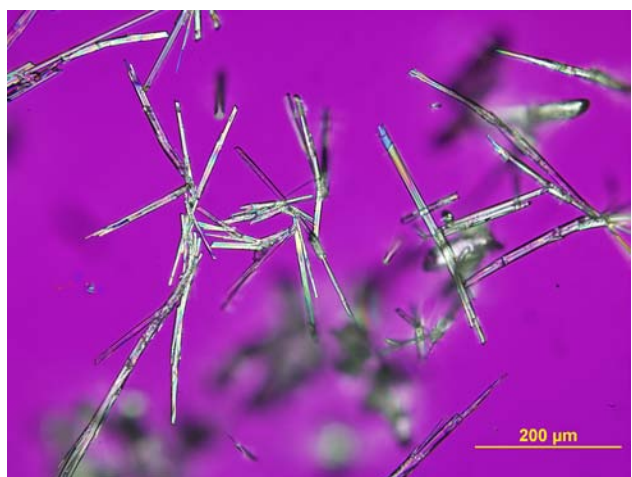


Figure 2-11. Salicylic Acid sublimates, crossed polarizers, first-order red compensator, 200X

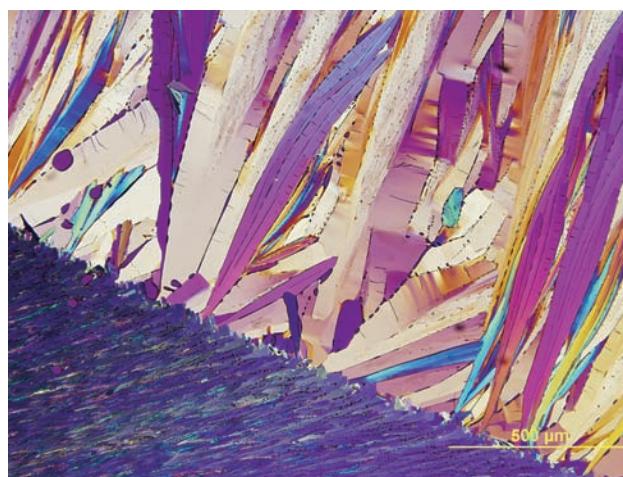


Figure 2-12. TNT polymorphs, crossed polarizers, first-order red compensator, 100X

18. Fluorescence: Color and intensity of fluorescence under ultraviolet 365 nm, (see Figure 2-14) and, possibly, blue-violet 400 nm excitation, or other excitation wavelengths.

There are many other characteristics of particles which may be determined, but those discussed above are usually all that are needed, and, in most cases, fewer characteristics are required to identify a substance. Now, it turns out that the first eight characteristics listed can be determined with any kind of microscope. Refractive indices, pleochroism, and dispersion staining all require that the sample be illuminated with light vibrating in one direction only — the “plane-polarized light” condition. Birefringence, sign of elongation, extinction angle, and interference figures all require that the sample be viewed between

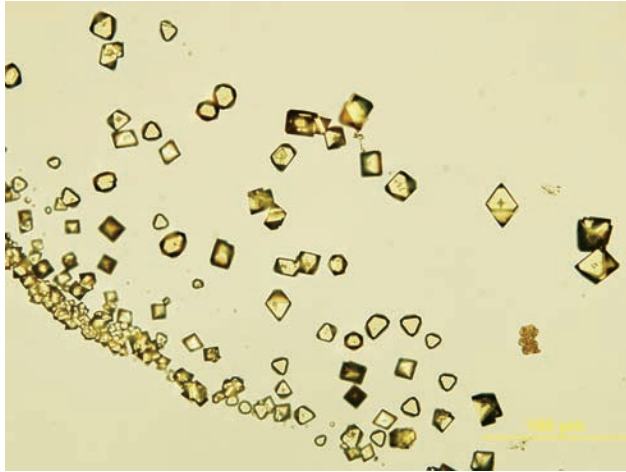


Figure 2-13. Alum, fully crossed polarizers; microchemical preparation with chloroplatinic acid for the detection of potassium ions, 400X.

two polarizers whose vibration directions are crossed (90°) relative to one another—the “crossed polarizers”, “fully crossed polarizers”, “crossed polars” or, formerly, “crossed Nicols” arrangement. Additionally, observations during heating and cooling of the particle, and certain microchemical tests, require crossed polarizers.

The only kind of microscope that will enable us to make *all* of the observations necessary for identification is a true polarized light microscope (PLM), or, simply, polarizing microscope. A phase contrast microscope, a reflected-light (metallurgical) microscope, and a biomedical, clinical-type microscope fitted with polarizing filters, as are commonly provided, are inadequate. The microscope must be a true polarizing microscope, as will be described later in detail.

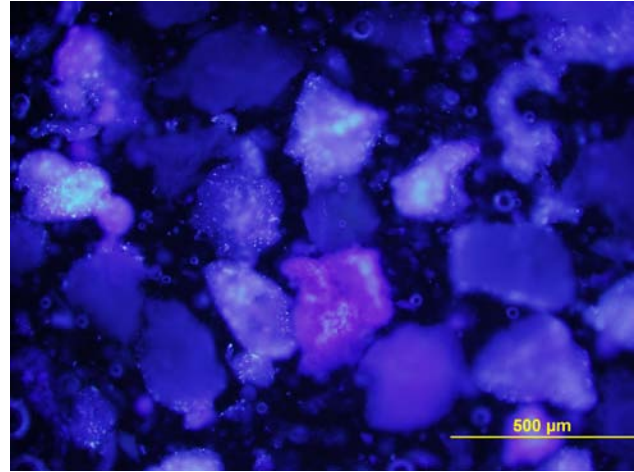


Figure 2-14. Laundry detergent, narrow-band 365 nm ultraviolet fluorescence, 100X.

3. PARTICLE IDENTIFICATION: OBSERVATIONS MADE USING ORDINARY LIGHT

Once experience has been gained in particle identification, the first view of a particle will be made using slightly uncrossed polarizers, but to begin with, it is a good idea to start systematically using ordinary light (see Figure 3-1). If a polarizer is in place, it may be left in the light path, as its presence does not affect the observations to be made. Another good idea to start with is to make a chart with particle types listed down the left side, and several columns headed with the titles: morphology, size, absorption color, pleochroism, refractive indices, dispersion staining, birefringence, sign of elongation, extinction angle, interference figure, fluorescence, and remarks. This will form a sort of checklist to ensure that all of the essential characteristics for identification are observed and recorded.

A. Morphology

To begin with, one of the first features that will be apparent on looking at a particle is its shape. Spheres, needles, flakes, plates, all will be immediately apparent. Describe in brief terms what is seen; include surface markings, textures, inclusions, degree of roughness or smoothness, sharp or rounded edges, type of fracture (conchoidal, rhombohedral, etc.), irregular or other outline in projected aerial view, cross-sectional shape of a fiber, ribbon, etc. Use as many descriptive terms as necessary to build up a word picture of the particle shape. Many organized biological structures (diatoms, pollen grains, plant hairs, insect parts) have characteristic morphology, and will soon become recognizable on sight; minerals, industrial dusts, and “white powders” will require a few more observations.

B. Size

Next, measure the size or dimensions, or length-to-width aspect ratios of particles using the calibrated eyepiece micrometer. Many biological structures are identified through various keys in which their diameter, distance between structures, etc., is required. If there is a size range, measure the smallest and largest particle of the same type, and record the mode as well. If the particles are irregular, determine Martin’s diameter (the dimension, parallel to the eyepiece scale, that divides a randomly oriented particle into two projected areas). If a particle size distribution is needed, tabulate the particles into predetermined size classes, and count enough particles so that commonly used statistical methods may be applied to the data.

Specific examples of each of these may be found in texts on quantitative polarized light microscopy [McCrone, McCrone, and Delly (1978)], and in texts devoted exclusively to particle size determination.

C. Absorption Color

Record the color, or range of colors seen, by both transmitted and reflected light. The “color by reflected light” brings up a brief diversion. I find it necessary to make a small modification to any microscope dedicated to particle identification, and that is the addition of a bifurcated fiber-optic illuminator for “top light”. Arrange the two flexible arms so that they point downward toward the specimen. To conserve bench space, the transformer may be mounted on a small shelf beneath the bench and to the rear so that it is out of the way. The light is controlled by a momentary-contact foot switch; the regular transmitted microscope light is also controlled by a foot switch of the on/off kind. The voltage is always at the maximum (12 V), an 80-A filter is in the light path, so that the color temperature of the light is always at 5500 K (daylight); different manufacturers may have an equivalent way of achieving daylight conditions. With Olympus, this is achieved at a voltage setting of 9 V, plus the use of an LBD filter in the light path. Intensity is controlled by neutral density filters. Now, when colors need to be observed, either transmitted or toplights or both can be turned on and off without letting go of the slide, focus, etc., and all colors will be recorded under standard daylight conditions.



Figure 3-1. Salicylic Acid, plane-polarized light, 100X

D. Magnetism

This is also a good time to take a small, strong magnet (of the kind mounted at the top end of carbide scibers) and wave it back and forth around the lower end of the objective; move the slide around with the other hand; occasionally turn the toplight on with the foot switch. Anything magnetic will “wave back” at you while you are viewing it, and you can note the relative percentage of the sample that is magnetic, and whether the magnetic particles appear scalelike, as wear particles, or if they show machining, drilling, filing, or shear marks; whether there is corrosion present, or evidence of rust, or mill scale. The degree of reflectivity; or are they round, or as magnetite spheres from fly ash, or grinding operations. All of these questions are asked and answered while the magnet is continuously moved back and forth, and different parts of the sample are viewed. This, of course, assumes that the sample is mounted in a refractive index liquid. .

E. Brittleness/Elastomericity

The brittleness or elastomericity of a particle may be determined in the following way: hold the slide with one hand, controlling the field of view, and with the other hand hold a dissecting needle and direct its point under the objective (start with a 10x objective).

You will see the needle’s shadow as you move it back and forth under the objective. Stop when it is in the middle of the field of view, and withdraw it until the point is centered in the field. Now move the particle in question to the center of the field, and depress the coverglass with the needle point directly above it. Observe how the particle responds to the depressed coverglass. Some particles, like diesel soot, carbon black, and iron oxide agglomerates, will immediately disintegrate into a “zillion” tiny ($< 1 \mu\text{m}$) particles. Others, like petroleum that has had the volatiles driven out, but is not yet fused, will be glassy, and will shatter when the coverglass is depressed. Still others, such as rubber from an enclosure, or from automobile tires, or from a conveyer belt, will flatten out easily, but will “bounce back” when the pressure is released (elastomeric). Elastomeric materials which have been degraded, as through thermal or ultraviolet influence, will not “bounce back”, but will “squooosh” and remain flattened. Perhaps there will be no change. All of these observations must be recorded and added to those already observed, as possibilities for identification are already forming in your mind as you integrate all of your observations.

4. PARTICLE IDENTIFICATION: OBSERVATIONS MADE USING PLANE-POLARIZED LIGHT

When you have learned all you can by observing the particle using ordinary light, it is time to check for pleochroism and refractive index, using Becke line, and dispersion staining methods.

A. Pleochroism

Pleochroism (“many colors”) is a change in color or hue of a colored, anisotropic particle relative to the vibration direction of plane-polarized light. Colored crystals which are anisotropic (have more than one refractive index) often exhibit different degrees of light absorption in different directions within the crystal, resulting in the crystal showing a different color, or different intensity of color, when rotated in plane-polarized light. Ordinary light will not do for *microscopic* specimens, because the light vibrates in all directions, and therefore the crystal will show a composite of all effects, which is analytically useless. Light vibrating in only one direction is required. The procedure is simple. If the transparent particle being viewed is colored, rotate the stage at least 90°, (alternatively, the polarizer may be rotated 90°, but it is usual to rotate the stage) and watch for any change in color or intensity. Record any changes. If the particle is elongated, the color changes seen are referred to the shape. For example, the common asbestos fiber, fibrous riebeckite is characteristically blue to blue-green in absorption color. If the fiber length is oriented east/west, i.e., parallel to the polarizer vibration direction, it will appear a blue or green color (see Figure 4-1), and this color is characteristic of the fiber length (n_{\parallel}), i.e., parallel to the polarizer vibration



Figure 4-1. Fibrous Riebeckite, plane-polarized light, polarizer East/West - fiber length East/West, 100X

direction, and is recorded as, n_{\parallel} blue, and read as “n parallel, blue”. The stage is then rotated 90° so that the fiber is now oriented north/south. The color of the fiber is now gray (see Figure 4-2), and this color is characteristic of the fiber’s width (n_{\perp}), i.e., the width is now parallel to the polarizer vibration direction, and is recorded as n_{\perp} gray, and read as “n perpendicular, gray”. In the column under pleochroism, this fiber would be recorded as n_{\parallel} blue; n_{\perp} gray.

Kevlar® fibers show n_{\parallel} pale yellow-green; n_{\perp} colorless. Colored anisotropic substances do not necessarily show detectable pleochroism, however, which is what makes its recorded observation an identifying characteristic.

Pleochroism can also be observed in *macroscopic* crystals using ordinary (not plane polarized) light; e.g. iolite and tourmaline can be diagnosed for pleochroism by rotating the crystal on an axis *not* perpendicular to the stage.

B. Refractive Index

Refractive index is the ratio of the velocity of light through a substance relative to the velocity in a vacuum. For example,

$$\frac{\text{velocity of light in vacuum}}{\text{velocity of light in glass}} = \frac{3 \times 10^{10} \text{ cm/s}}{2 \times 10^{10} \text{ cm/s}} = 1.5$$

Notice that there are no units. Refractive index is expressed as n for cubic crystals and amorphous

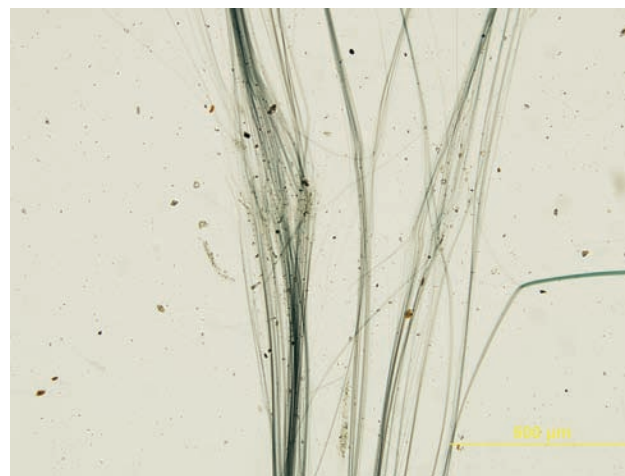


Figure 4-2. Crocidolite (fibrous Riebeckite), plane-polarized light, polarizer East/West - fiber length North/South, 100X

substances; thus, the refractive index of glass is expressed as $n = 1.5$. If the refractive indices of all solids and liquids were plotted against the number of materials with those indices, there would be formed an approximate bell-shaped curve, in which the lowest refractive index would be around $n = 1.3$ (gases have lower indices; the refractive index of air is $n = 1.0$). Sodium fluoride is $n = 1.32$; water is $n = 1.33$; ethanol is $n = 1.34$; my favorite cognac is $n = 1.3568$ (one has to know the important things!). The peak of the curve would fall around $n = 1.52$. Diamonds are $n = 2.419$, and the oxides of the heavy metals range from $n = 3$ to $n = 5+$. The exact value depends on the crystal's atomic makeup, and is, therefore, different for each different material. Our aim then is to determine the characteristic identifying values of these refractive indices for each material.

Liquids have one refractive index, designated n . Isometric cubic crystals also have one refractive index, designated n . Tetragonal and hexagonal (including trigonal) crystals have two refractive indices, designated ϵ and ω , either one of which may be the higher value, i.e., $\epsilon > \omega$ or $\epsilon < \omega$. This provides for a further subdivision of all tetragonal and hexagonal crystals; namely, those in which $\epsilon > \omega$ which are, by convention, termed "optically positive" (positive sign of double refraction), and those in which $\epsilon < \omega$, which are termed "optically negative" (negative sign of double refraction). Orthorhombic, monoclinic and triclinic crystals have three refractive indices, designated α , β , γ . By definition, α is the lowest refractive index, γ is the highest refractive index, and β is intermediate in refractive index. Note that β , being intermediate, can be closer to α or closer to γ . This allows for the further subdivision of all orthorhombic, monoclinic, and triclinic crystals into two subclasses: If $\gamma - \beta > \beta - \alpha$, the crystal is said to be "optically positive"; if $\gamma - \beta < \beta - \alpha$ the crystal is said to be "optically negative".

In principle, the determination of the refractive index is simple. Consider the case of a cubic crystal, such as ordinary sodium chloride ($n = 1.5443$). If this crystal were placed in a liquid whose refractive index is also $n = 1.5443$, the solid would be invisible. Thus, given an unknown cubic crystal, one places it in one of the refractive index liquids in the $n = 1.3 - 1.7$ series and compares it to the medium through the relief that is produced. The liquids are changed until one is found in which the crystal visually disappears. Then, knowing the refractive index of the liquid, the refractive index of the crystal is known, and one then goes

to a table of refractive indices of cubic crystals, where the value $n = 1.5443$ will be found next to sodium chloride.

In practice, there are a couple of things that must be taken into consideration. One of these is that the refractive index varies with wavelength, being, generally, higher for blue than for red; this is referred to as normal dispersion. [It should be noted, however, that in the case of certain colored materials in which an absorption band is present, the refractive index will actually increase with the wavelength within the absorption band, before again decreasing—this phenomenon is referred to as anomalous dispersion]. In practice, a single standard wavelength has to be agreed upon to record refractive index values. The wavelength selected for the standard is 589 nm, Fraunhofer's D line due to sodium, which produces an orange color (see Figure 4-3). This wavelength is commonly obtained through the use of an interference filter of 589 nm, where the filter is placed over the light exit port of the microscope. Inexpensive substitutes for interference filters are available, but for professional use the interference filter is recommended.

Another consideration to keep in mind is that temperature affects the refractive index. As a substance is heated, it expands, there is more space between the structural elements, light travels faster, and the refractive index decreases. As a substance is cooled, it contracts, there is more matter in the path of the light and it slows down; the refractive index increases. Thus, some standard temperature must be agreed upon for the expression of refractive index. In the United States, the standard is 25°C; in Europe the standard is 20°C. The correct designation for sodium chlo-

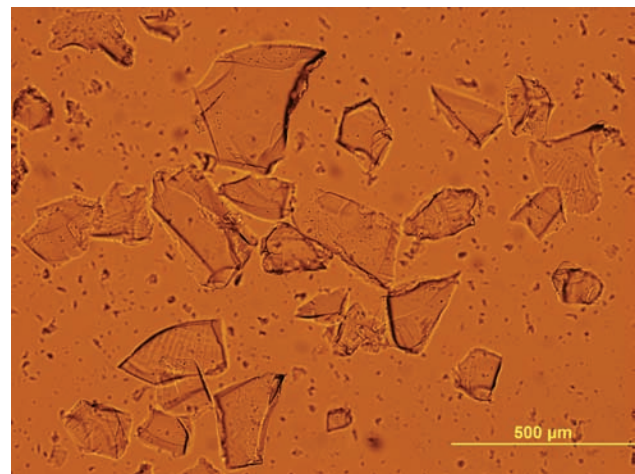


Figure 4-3. Silica gel, plane-polarized light, 589 nm sodium D interference filter, 100X

ride, for example, is $n_D^{25^\circ\text{C}} = 1.5443$. A thermometer is provided with all complete sets of refractive index liquids so that the temperature may be determined. Normally, the bulb of the thermometer is placed on the microscope stage in place of the specimen where the liquids will be used; this is done to take into account the increase in temperature due to local heating from the light source.

In the event that the temperature is not 25°C , or if the liquids are used in a hotstage or coldstage, a temperature correction must be applied. There is a temperature coefficient indicated on each bottle of refractive index liquid. A typical one reads $-dn/dt = 4 \times 10^{-4}/^\circ\text{C}$. That is, there is a change in refractive index (dn) that amounts to 0.0004 refractive index units for each 1°C change in temperature (dt). Suppose one is using liquid $n_D^{25^\circ\text{C}} = 1.680$, and the thermometer on the stage reads 21°C . That is a 4°C difference from the standard 25°C : The liquid changes index by 0.0004 unit for each degree off of standard, or $4 \times 0.0004 = 0.0016$, and since it is *colder* than standard, the liquid will contract, and the index will *increase*. Therefore the 0.0016 is *added* to the bottle's value of 1.680, or $n_D^{25^\circ\text{C}} = 1.6816$.

For laboratories with constant temperature doing routine work, the correction is seldom necessary, but it is essential for any patent-related or legal work.

If on placing an unknown crystal in a liquid and the crystal and liquid do not match, it is essential to determine which is higher or lower so that one knows in which direction to go to find a matching liquid. This is done through use of the Becke line. The Becke line, named after F. Becke, an Austrian mineralogist, is a halo that forms around a particle as the result of using axial illumination, achieved by closing the aperture diaphragm—which also reduces the illumination level; geologists are taught to flip-out the top lens of the sub-stage condenser. Normally one would not want to close this diaphragm because of the loss of resolving power. However, the resolving power is not relevant here; the halo is. The Becke line halo is best produced with high numerical aperture objectives, usually the $40\times/0.65$, with a 589 nm filter in place, if the monochromatic light is desired, and the particle is brought into good focus. Now, the fine adjustment is suddenly turned in such a way that one is focused *above* best focus (on Olympus microscopes this means turning the fine focus *toward* or counter-clockwise to the operator, which *lowers* the stage, which is the same as focusing slightly above

the sharpest or best focus, i.e., higher than the plane of the specimen). During this operation, the Becke line will be seen to move either into the particle or into the medium. On focusing above the best focus like this, the Becke line *always* moves toward whichever medium, the grain or liquid that has the higher index (“focus high, goes high”). If one focuses *below* best focus, the opposite effect occurs. In practice, one focuses both ways to confirm the “up” observation. Start at best focus, and turn toward you, and away; toward you, and away; up, down; up, down, up. Stop at “up” and decide which way the halo of light is moving. If the Becke line moves into the particle, the particle has the higher index, and a higher liquid must subsequently be chosen to match the particle's refractive index. If the Becke line moves into the liquid, the liquid is higher in index, but we are interested in the particle: if the liquid is higher, the particle is lower in refractive index. The amount of relief or contrast will give a visual estimate of the refractive index difference. If the contrast is great, choose a new liquid many bottles away. It is possible to obtain the refractive index value using no more than three bottles.

If there is very little contrast, and the particle is difficult to see, you are close (see Figure 4-3); choose a liquid, say, two bottles away.

C. Dispersion Staining

There are several ways of obtaining dispersion staining colors, including the use of phase contrast objectives, darkfield stops, and specialized dispersion staining objectives. Here, we will describe the specialized dispersion staining objective, because that is what is supplied on the microscopes at the College of Microscopy. A special objective is required for this technique: it may be one which has a centerable, central light-occluding stop, or one in which the stop is permanently mounted in the objective back focal plane.

The dispersion staining objective is a $10\times$ objective, and the central stop is by far the most useful, as it gives a black background against which the dispersion staining colors stand out brilliantly. To use the objective, focus on a sample in the normal manner, then swing out the top lens of the condenser to lower the numerical aperture. Put in the Bertrand lens, at which time you will see the central stop in the objective's back focal plane. Close the aperture diaphragm until its image almost touches the central stop. If the central stop is not exactly centered within this closed aperture diaphragm, center the stop with the

centering wrenches, or the centering screws depending on the model of dispersion stainer used; today, unfortunately, dispersion-staining objectives may be supplied with fixed, non-centerable center stops. When the stop is centered, remove the Bertrand lens, and, while viewing the specimen, close the aperture diaphragm slowly until the background just becomes black. If the dispersion curve of the solid crosses the dispersion curve of the liquid anywhere in the visible portion of the spectrum, the particles will be seen in brilliant colors against a black background (see Figures 4-4, 4-5, 4-6, and 4-7). If the dispersion curves of the solid and liquid cross outside of the visible region, the particles will appear white against a black background.

The colors seen in dispersion staining depend on the refractive index relationship between the solid and the liquid, so that the colors observed can be used to tell what the refractive indices of the solid are, without having to prepare multiple immersion mounts for doing the Becke line test. It is a very rapid way of obtaining refractive index data on many particles at once.

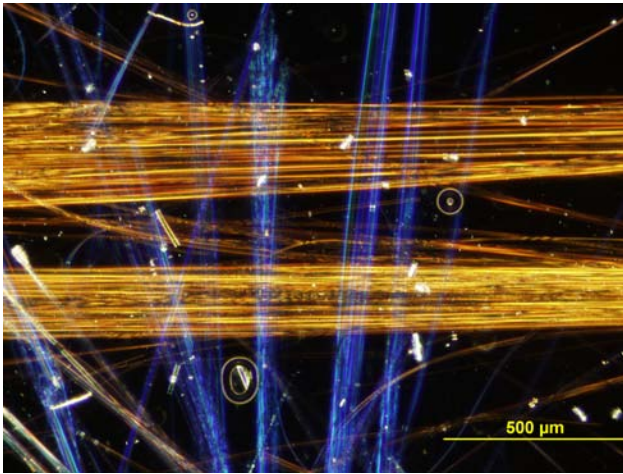


Figure 4-4. Amosite, dispersion staining, polarizer East/West, 100X

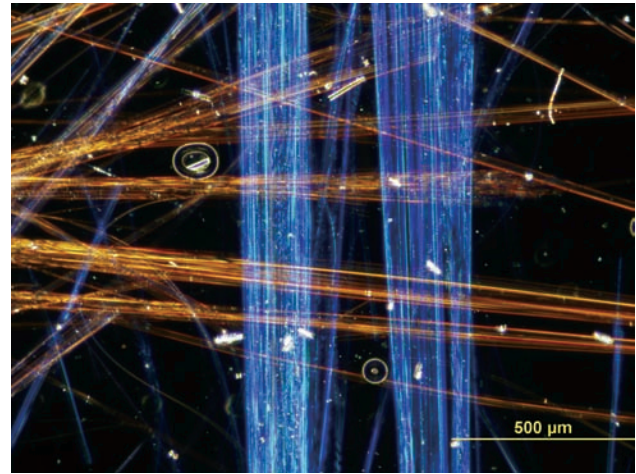


Figure 4-5. Amosite, dispersion staining, polarizer East/West, sample rotated 90°, 100X

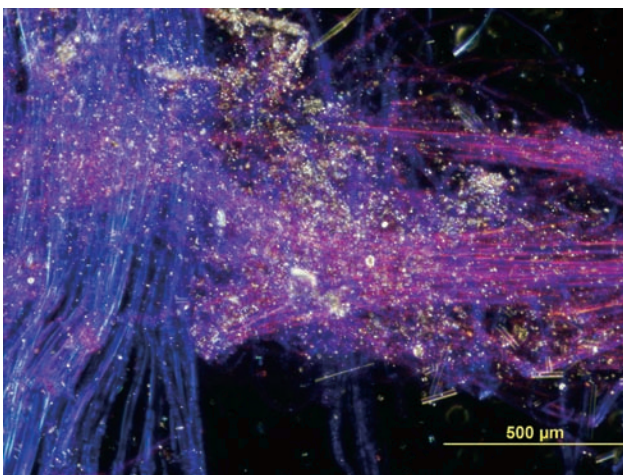


Figure 4-6. Chrysotile, dispersion staining, polarizer East/West, 100X

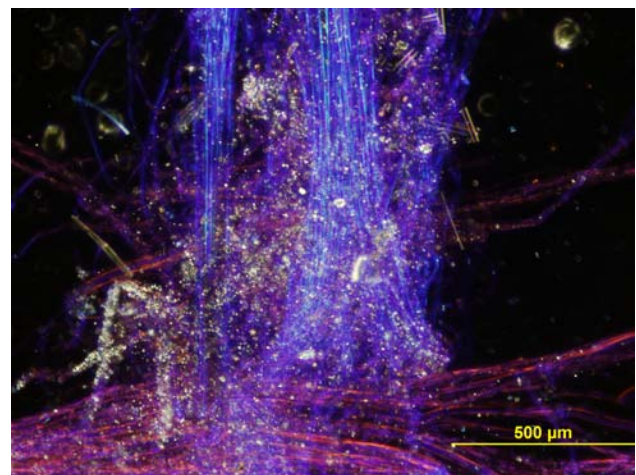


Figure 4-7. Chrysotile, dispersion staining, polarizer East/West, sample rotated 90°, 100X

5. Particle Identification: Observations Made Using Crossed Polarizers

A. Birefringence

Birefringence is the numerical difference between the maximum and minimum principal refractive indices. For amorphous materials and crystals in the cubic system, the birefringence is zero, because all of these materials have one refractive index only. For tetragonal and hexagonal crystals, the birefringence is $\varepsilon - \omega$ (the absolute value, since either may be the larger value). For orthorhombic, monoclinic, and triclinic crystals, the birefringence is $\gamma - \alpha$. Birefringence, therefore, may be either $\varepsilon - \omega$ or $\gamma - \alpha$, and the general form of the equation is $\delta = n_2 - n_1$ where n_2 and n_1 represent $\varepsilon - \omega$ or $\gamma - \alpha$, depending on the symmetry crystal being examined. The birefringence may be determined through use of the refractive indices obtained via the Becke line method or dispersion staining, but there is a much faster way that is based on the particle's thickness and the interference color it shows between crossed polarizers due to its retardation.

When a plane-polarized beam of light enters an anisotropic crystal it is immediately divided into two components, one traveling in the fast direction and one traveling at a reduced velocity in the slow direction (see Figure 5-2 on following page). The two components therefore exit the crystal at different times; the fast component leaves first, and the slower component follows behind. The slow component is said to be “retarded” relative to the fast component, and the physical distance between the two, measured in nanometers (nm), is known as the retardation. The exact value of the retardation depends on the numerical difference between the refractive index of the fast and slow rays (birefringence), and on the crystal's thickness. Specifically,

$$\Delta = 1000 \delta t,$$

where Δ is the retardation (measured in nanometers), δ is the birefringence, t is the thickness (measured in micrometers), and the 1000 is a conversion factor necessary to convert the thickness in micrometers into nanometers, the unit of retardation. By transposition, we obtain:

$$\Delta = 1000 \delta t$$

$$\delta = \Delta/1000t$$

$$t = \Delta/1000\delta$$

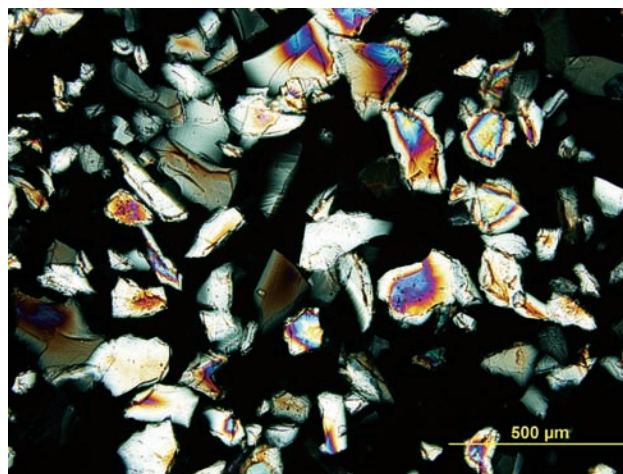


Figure 5-1. Quartz, crossed polarizers, 100X

Normally, we do not obtain thickness through calculation, because it is estimated from other particle measurements made with the calibrated eyepiece micrometer. The retardation is obtained from the color that the particle shows between fully crossed polarizers. The colors are those in the Newtonian series, and from a chart of these colors, known as the Michel-Lévy chart, the number of nanometers corresponding to a particular color may be read directly. It is the birefringence that we are normally after, as this is a fundamental characteristic of materials. Quartz, for example, has a birefringence of 0.009, this is the numerical difference between its two refractive indices, and constitutes one of its identifying characteristics. The birefringence is constant. The retardation color that quartz shows between crossed polarizers will vary with the thickness and orientation, and so a field of quartz grains is typically quite colorful because of the variations in thickness and orientations among the different grains (see Figure 5-1).

Retardation (measured in nanometers) results from plane-polarized light passing through a crystalline material that has more than one refractive index (see Figure 5-2). “Slow” is the high refractive index direction; “Fast” is the low refractive index direction. The slow ray here is retarded relative to the fast ray on exiting the crystal.

To obtain the birefringence of an unknown particle, we need to know its thickness and retardation. The thickness is obtained through direct measurement or estimation from other measurements using the calibrated eyepiece micrometer. The retardation is obtained by matching the color at the point where the thickness is determined with the color on the Michel-Lévy chart (see Figure 5-3), and reading the value in nanometers. The Michel-Lévy chart is actually a graphical solution to the birefringence equation up to the limits of 50 μm for thickness, or about 1800 nm for retardation, $\delta = \Delta/t1000$.

$$\text{Birefringence } (\delta) = \frac{\text{retardation (nm)}}{\text{thickness } (\mu\text{m}) \times 1000}$$

In actual practice what is done is this: The particle of interest is viewed between crossed polarizers and oriented to its maximum brightness position. Ideally, a grain showing maximum δ (based on the number of isochromes) is chosen, then rotated to its maximum brightness. Next, its thickness is estimated by taking linear measurements with the calibrated eyepiece micrometer, and its maximum retardation color is noted. The *maximum* color is noted, because a three-dimensional grain will show a *series* of isochromes, in contrast to a thin section, where a grain shows only a single color due to its planar shape (constant thickness throughout). Going to the Michel-Lévy chart, one enters at the thickness, moves along this thickness line to the right until one gets to the retardation color (vertical line) that the particle is showing. A diagonal line is the intersection of the thickness and retarda-

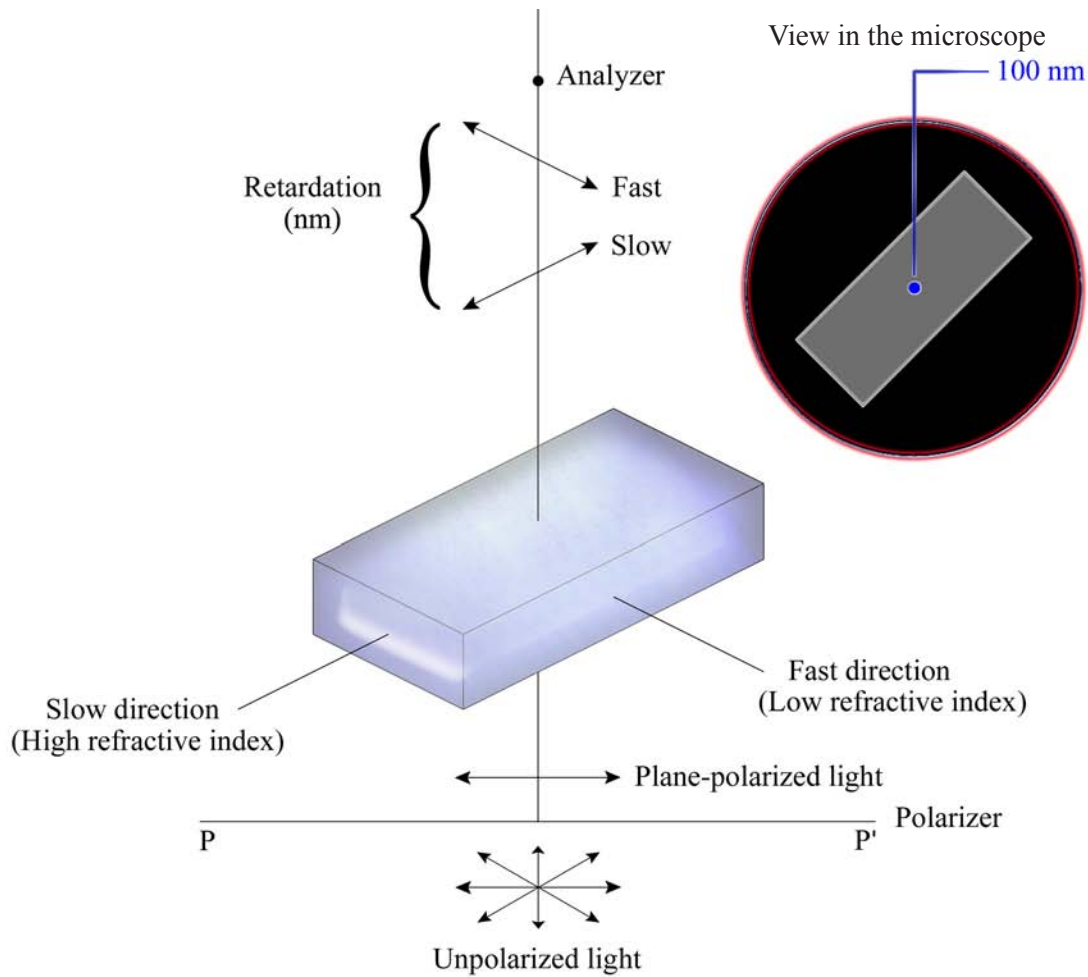
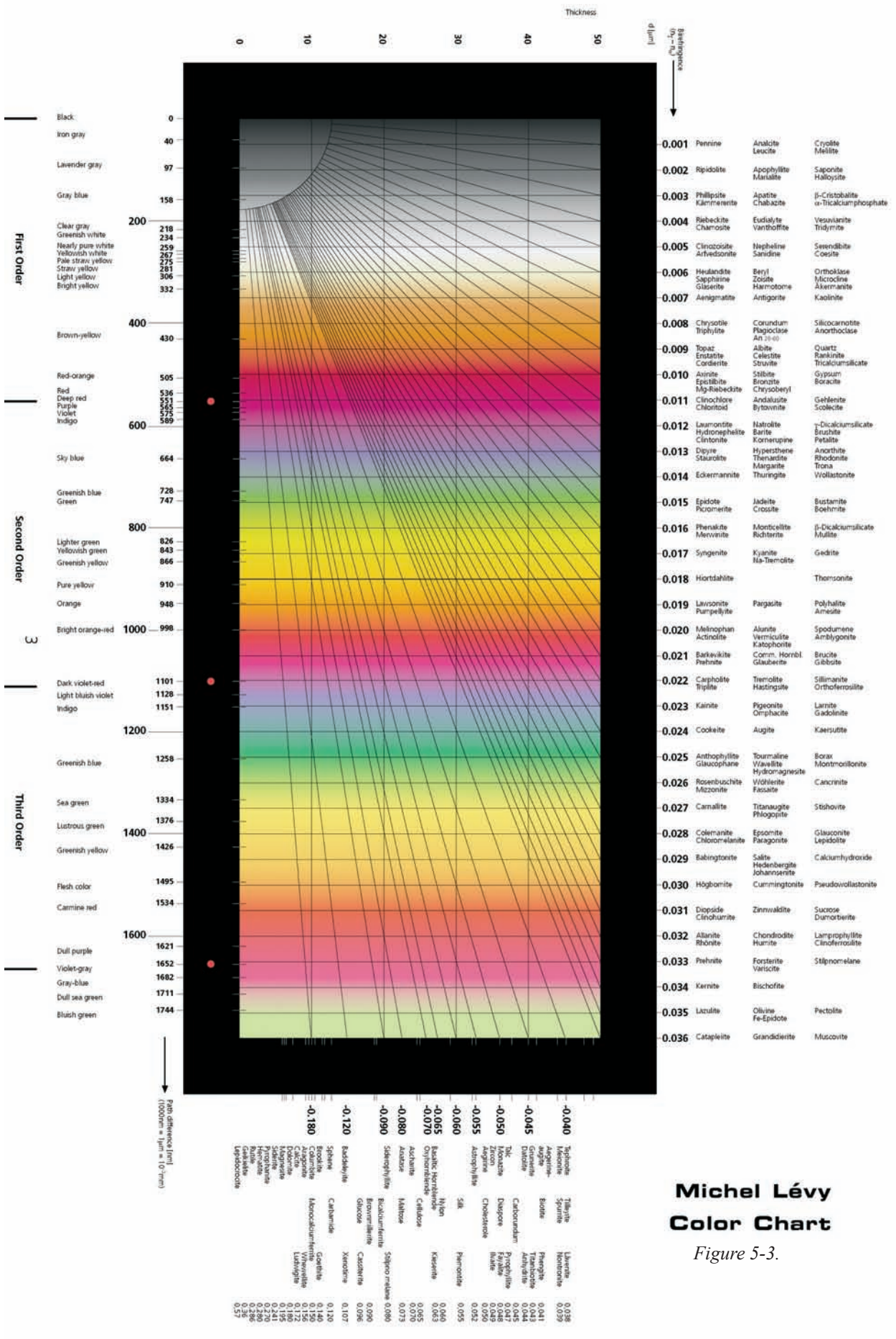


Figure 5-2. An anisotropic crystal oriented 45° to the vibration plane of plane-polarized light; the slow (high refractive index) and fast (low refractive index) directions are indicated.



**Michel Lévy
Color Chart**

Figure 5-3.

tion. Follow the diagonal line up and to the right, and read the birefringence. Of course, equations may be used to calculate the birefringence, but using the chart is much faster. For thickness and retardation beyond those indicated on the chart, one simply extrapolates. Further details on the use of this chart, including a history, can be found at www.ModernMicroscopy.com.

GLOSSARY OF TERMS

Anisotropic

(an-: not; iso-: same; -tropos: velocity)
Refers to substances that have more than one refractive index, such as crystals in the hexagonal or tetragonal system (two principal refractive indices), orthorhombic, monoclinic, or triclinic system (three principal refractive indices), oriented polymers, or isotropic substances that are mechanically or thermally strained. Anisotropic substances can be seen between crossed polarizers, if they are not in an extinction position. Compare Isotropic.

Becke Line

A band or halo of light (due to diffraction/refraction) seen at the periphery of a specimen when the refractive indices of the specimen and its mounting medium are different; it is used to determine refractive index. In practice, the Becke Line is produced by reducing the numerical aperture of the substage condenser, and focusing above and below the plane of best focus; the Becke Line always moves toward the material of higher refractive index on focusing above the plane of best focus. Named after the Austrian geologist, mineralogist, and petrologist, Friedrich Johann Karl Becke, 1855-1931, who devised the method.

Birefringence (Double Refraction)

The numerical difference between the maximum and minimum refractive indices of anisotropic substances: $\varepsilon - \omega$; $n_{\parallel} - n_{\perp}$ (“n parallel minus n perpendicular”; “applied in work with fibers”); $\gamma - \alpha$. Birefringence may also be determined by use of compensators, or estimated through use of the Michel-Lévy Interference Color Chart. Birefringence may be qualitatively expressed as low (0 - 0.010), moderate (0.010 - 0.050), or high (>0.050); often birefringence >0.2 is termed “extreme”. “Strain birefringence” is a term used to refer to isotropic substances which exhibit uneven (usually) or spotty birefringence induced by mechanical, thermal, chemical, or other means to induce strain and impart an anisotropic character to an otherwise isotropic material.

Cleavage

The quality or property of a crystallized substance, mineral, or rock of splitting, parting, or separating along definite crystallographic planes. Classic examples include the minerals calcite, gypsum, and mica.

Columnar

A term used to describe a shape resembling tall, narrow, somewhat cylindrical or prismatic crystals. In some traditional texts, the term is applied to aggregates.

Compensator

A device with a known, fixed or variable retardation, and vibration direction, used for determining the amount of retardation (hence, the thickness and birefringence) of an anisotropic substance. It is also used to determine the sign of elongation of elongated anisotropic specimens. The most common compensator is the first-order red (530-550 nm retardation), but other compensators frequently used include the quartz wedge (1-6 orders), the Berek (a tilting compensator, varying from 1-3, 1-5, 1-30 orders), the quarter-wave (~137 - 147 nm), and the Sénarmont. Compensators are typically introduced into the light path through a body tube slot located between the objective and the eyepiece.

Euhedral

Refers to well-formed crystals; those that are bounded by plane faces. The combining form eu – means “well” or “good”; the combining form – hedron means a “surface”. “Polyhedral” (poly – means “many”) refers to many faces. “Anhedral” (an – means “without”) refers to the absence of faces. “Subhedral” (sub – means “below/under”) refers to imperfectly developed faces.

Equant

A shape having equal or nearly equal dimensions or diameters in all directions (equidimensional). The term is applied to crystals, spheres, sub-spheres, or rounded particles of any kind, such as spray-dried materials and water-worn/wind-worn sand; that is, a particle approaching or approximating a spherical shape.

Extinction

The condition in which an anisotropic substance appears dark when observed between crossed polarizers. This occurs when the vibration directions in the specimen are parallel to the vibration directions of the polarizer or analyzer. Extinction may be complete or incomplete; common types include parallel, oblique (inclined), symmetrical, and undulose.

Extinction Angle

The angle between the extinction position and some prominent length, face, or feature of an anisotropic specimen. In practice, it is the numerical difference between the stage readings taken (1) when the specimen's prominent length, face or feature is aligned with the eyepiece crosshair, and (2) then rotating (clockwise/counter-clockwise) to the extinction position; when angles are being measured for elongated specimens it is customary to report the smaller of the two observed angles, but only because that is usually the z^c (not always; needs to be checked with a wave plate).

Fluorescence

The luminescence (low-temperature emission of light) that is caused by the absorption of radiation at one wavelength (excitation wavelength), followed by nearly immediate re-radiation usually at a different (longer) wavelength, and that ceases almost at once when the excitation wavelength stops (as opposed to phosphorescence, in which the re-radiated wavelength persists, often for a relatively long time, after the excitation wavelength stops. In microscopy, fluorescence may be primary, in which the sample is untreated, or secondary (induced) in which fluorochromes are introduced into the sample, as is the case with most biomedical specimens. In microscopical particle identification, the excitation wavelength is almost always in the ultraviolet region (narrow band or wide band 365 nm); any fluorescence remaining after the excitation wavelength is removed by barrier filters, and is reported as to its intensity and color(s).

Hardness

Hardness is a good, useful physical property to use in mineral identification and description. About 170 years ago, the German mineralogist Friedrich Mohs devised a ten-point scale of hardness using common minerals ranging from soft talc (1) to diamond (10). It is today universally used as a way of distinguishing different minerals. Specifically, the minerals and their corresponding ordinal-scale hardness are:

1. Talc
2. Gypsum
3. Calcite
4. Fluorite
5. Apatite
6. Orthoclase
7. Quartz
8. Topaz

9. Corundium (ruby-sapphire)

10. Diamond

A set of these minerals is typically assembled as a "hardness kit". These minerals are often supplemented by scratching an unknown mineral with a fingernail (hardness 2.5), an old copper penny (3.5), a piece of window glass or pocket knife blade (5.5), and a streak plate or steel file (~6.5).

As a memory aid to remembering the correct order of the minerals in Mohs' scale, several mnemonics have been devised, including: Those Girls Can Flirt And Other Queer Things Can Do, and Tom Gave Cathy Flowers And Ordered Quail To Cook Dinner.

Hexagonal

(hex-six; -gon knee; angle)

Relating to a crystal system characterized by three equal lateral axes intersecting at angles of 60 degrees, and a vertical axis of variable length at right angles. Crystals in the hexagonal crystal system have two principal refractive indices ϵ and ω ; ϵ may be greater than or less than ω .

Interference Colors

The colors seen due to the effect of the interaction of two mutually perpendicular wave trains of light that travel through an anisotropic material. In microscopy, these are the Newtonian series of colors seen when observing samples of more than one refractive index (i.e., anisotropic) between crossed polarizers in non-extinction orientations.

Isotropic

(iso-: same; -tropos: velocity)

Refers to substances that have only one refractive index, such as gases, glasses, liquids (except liquid crystals), unoriented polymers, and crystals in the isometric (cubic) system. Isotropic substances cannot be seen between crossed polarizers, regardless of their orientation. Compare Anisotropic.

Lamellar

A lamella (plural: lamellae) is a thin, flat, plate, scale, membrane, or layer. "Lamellar" is used to describe anything composed of such lamellae.

Lath-like

A shape referring to a thin, narrow, and long structure, with the relative dimension of a yard-stick or meter-stick. Compare Acicular.

Monoclinic

(mono-: one; -clin: incline)

Relating to a crystal system characterized by three unequal axes, one of which is inclined (oblique) to the plane formed by the other two. Crystals in the monoclinic system have three principal refractive indices α , β , γ .

Optic Axial Angle (2V)

(axial angle; optic angle; 2V)

The angle between the optic axes; that is, the angle between the normals to the circular sections of a biaxial ellipsoid; the symbol for the angle is 2V.

Where two such angles sum to 180°, the acute angle is understood to be designated “2V”; it is an important identifying characteristic of orthorhombic, monoclinic, and triclinic crystals.

Orthorhombic

(ortho-straight, right, true; - rhombic: a parallelogram with four equal sides)

Relating to a crystal system characterized by three unequal axes at right angles to each other. Crystals in the orthorhombic system have three principal refractive indices, α , β , γ .

Platy

The shape of a crystal with one short dimension, and two longer, approximately equal dimensions; flat.

Pleochroism

(pleo-: more/plus/many; -chroic: color)

The change in color or hue of colored, anisotropic substances relative to the vibration direction of plane-polarized light. In practice, the colored, anisotropic specimen is observed using plane-polarized light, while the stage is rotated at least 90°; alternatively, the polarizer may be rotated relative to the fixed specimen. The change in color and relative intensity, which also depends on specimen orientation, are noted and recorded.

Refractive Index (Index of Refraction)

The ratio of the velocity of light in a vacuum relative to the velocity of light in a medium (solid, liquid, gas); it is expressed as n , and varies with wavelength (λ) and temperature (°C). A common standard temperature is 25°C (another is 20°C); a common standard wavelength is 589 nm (Fraunhofer D); a common precision used in microscopy is ± 0.002 (or ± 0.001 if combining drops from adjacent refractive index liquids differing by 0.002).

Example: $n = 1.528$

Rhomb

Rhomb = Rhombus = Rhombohedron. A rhombus is a parallelogram with four equal sides, and sometimes one with no right angles. “Rhomboid” is a parallelogram with no right angles, and with adjacent sides of unequal length. A rhombohedron is a parallelepiped whose faces are rhombuses. A parallelepiped is a 6-faced polyhedron all of whose faces are parallelograms lying in pairs of parallel planes. A parallelogram is a quadrilateral with opposite sides parallel and equal. See any comminuted calcite preparation for example.

Sign of Elongation

A term used to describe the orientation of the vibration directions of the slow and fast rays in an elongated, anisotropic substance. By convention, a specimen is described as positive (+) when the “slow direction” (higher refractive index) is lengthwise (“length slow”), and negative (-) when the “fast direction” (lower refractive index) is lengthwise (“length fast”).

Specific Gravity (s.g.)

The ratio of the density of a substance to the density of a substance, such as pure water, taken as a standard, when both densities are obtained by weighing in air. “Density” is the mass of a substance per unit volume, expressed, for example, as grams per cubic centimeter, etc.

Tetragonal

(tetra-: four; -gon: knee, angle)

Relating to a crystal system characterized by three axes at right angles to each other, of which only the two lateral axes are equal; the third axis may be less than or greater than the other two. Crystals in the tetragonal crystal system have two principal refractive indices ϵ and ω ; ϵ may be greater than or less than ω .

Triclinic

(tri-: three; clin-: incline)

Relating to a crystal system characterized by three unequal axes, mutually oblique, all three unequal axes intersect at oblique angles. Crystals in the triclinic system have three principal refractive indices, α , β , γ .

Twin (Twinning)

A compound crystal composed of two adjoining crystals, or parts of crystals, of the same kind that share a common plane of atoms. Microscopic crystals of sodium bicarbonate commonly display twins.

NOTE: For microscopical terms not found in this glossary, consult one of the following:

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See also: <http://resolution.umn.edu/glossary/>

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