The Michel-Lévy Interference Color Chart—Microscopy's Magical Color Key

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The MICHEL-LÉVY interference color chart is a color key to unlock many mysteries associated with particle analysis. The chart (Hooke College students receive a laminated version) is a valuable aid to the light microscopist in that it graphically relates the thickness, retardation (optical path difference), and birefringence (numerical difference between the principal refractive indices) for particular views of transparent, colorless, or lightly-colored substances. These characteristics allow unknown materials to be identified, or provide important optical information about those which are known.

Early applications of the Michel-Lévy interference color chart were in the fields of mineralogy and petrology for the identification of mineral grains, either comminuted (Figure 1) or in rock thin sections (Figure 2). Other examples include thermal fusion preparations (Figure 3) and crystallization from chemical solutions (Figure 4). Today, however, it is used chiefly as an aid in identifying minerals, synthetic textile fibers, chemicals, food processing ingredients, biologicals, abrasives, drugs, catalysts, ores, fertilizers, and combustion products. In fact, it is used routinely by analytical microscopists in the identification of almost all dust-sized particles regardless of nature or origin (1). Some specific fields of application include criminalistics, air pollution, pharmaceuticals, aerospace, microelectronics, papermaking fibers, and polymers. Colors caused by the interference effects of light are commonly seen in soap bubbles, thin oil films, and between two pieces of glass separated by a thin layer of air. The same kinds of interference colors are seen in most solid particles when the observation is made using a polarizing microscope. The polarizing microscope is, of course, a conventional microscope with the addition of polarizing elements above and below the specimen,

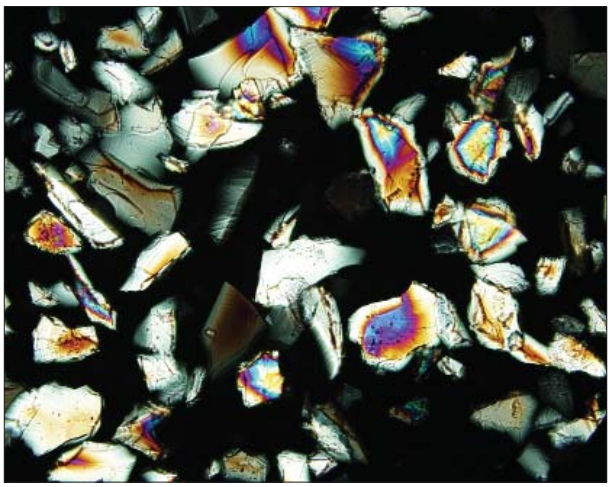

Figure 1
Comminuted quartz, crossed polarizers.

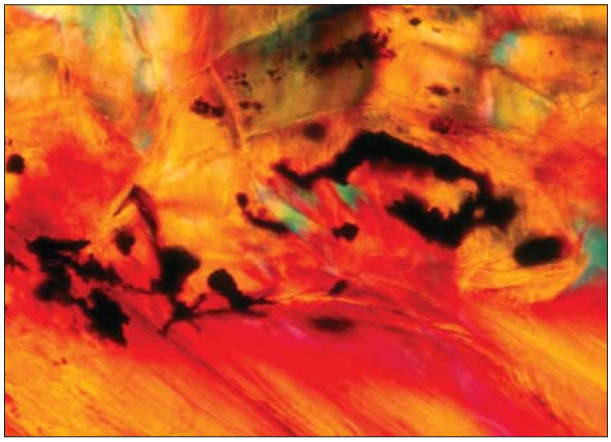

Figure 2
Serpentine thin section, crossed polarizers.

Figure 3
pp' DDT, crossed polarizers.

usually in the "crossed" position; however, biological type microscopes can be converted for interference color observation by placing one polarizing element, the "polarizer," anywhere below the specimen in the light path, such as in the filter carrier, between the lenses of the condenser, on the top lens of the condenser, or near the light source. The second polarizing element, the "analyzer," is placed in a "crossed" position above the specimen, such as on the back of the objective, the eyepiece, on the eyepiece diaphragm, or over the top lens of the eyepiece. A true polarizing microscope with graduated, rotating stage, slot for accessory plates, and other features is necessary for more-advanced, quantitative crystallographic work.

Color where there is none

What kinds of substances show interference colors? All transparent substances in the world, natural or man-made, when viewed in all orientations with the polarizing microscope, either show interference colors and appear bright and colored on a dark background, or cannot be seen at all (the field remains dark). Those substances which remain dark at all orientations between crossed polarizers are termed *isotropic*. They are either amorphous (e.g., glass) or they belong to the isometric (cubic) crystal system, and have just one refractive index; that is, light passes through the material at the same speed in all directions.

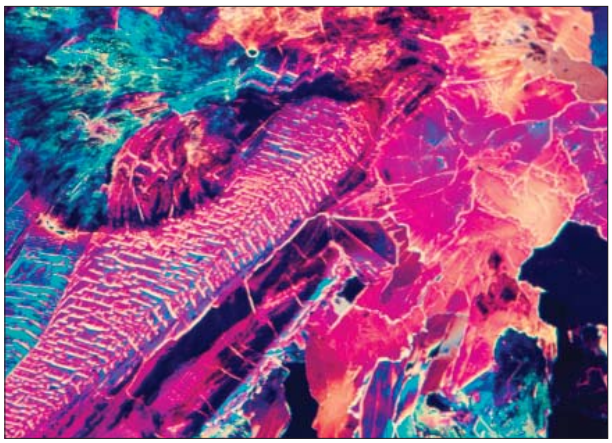

Figure 4
Chromium and ammonium oxalate, crossed polarizers.

To identify isotropic substances it is only necessary to determine the value of that refractive index (usually done by the Becke line, or immersion method) and look up the value in reference books where such data are tabulated. Examples of isotropic substances are common table salt, silver chloride, diamond, glass, and unoriented polymers.

Substances which appear bright with interferences colors on a dark background are termed *anisotropic*. Anisotropic substances which are uniaxial have two principal refractive indices, and belong to the tetragonal or hexagonal crystal systems. Examples of uniaxial substances are quartz, calcite, silicon carbide, synthetic textile fibers, and oriented polymers.

Biaxial anisotropic substances have three principal refractive indices, and belong to the orthorhombic, monoclinic, or triclinic crystal systems. Examples of biaxial substances are borax, talc, sucrose, mica, and gypsum. The refractive indices are characteristic identifying properties of transparent substances. It is these anisotropic substances which can be identified with the aid of the Michel-Lévy interference color chart, often without having to determine each principal refractive index individually.

The characteristic birefringence of a given substance is the numerical difference between the maximum and minimum refractive indices; that is $(\varepsilon - \omega)$ for uniaxial substances and $(\gamma - \alpha)$ for biaxial substances. We can designate birefringence generally as $(n_2 - n_1)$. Only in particular orientations of a crystal do we see the characteristic birefringence; all other orientations show lower values of $n_2 - n_1$.

When plain polarized light from the polarizing element below the specimen enters a doubly refracting (anisotropic) substance, it is resolved into mutually perpendicular slow and fast rays. Each of these rays passes through the crystal at its characteristic velocity, and both emerge from the top of the crystal with a path difference, the *retardation*, measured in nanometers (nm). When the two rays are recombined in the analyzer, this retardation, a phase shift, causes, to a greater or lesser degree, destructive interference for certain wavelengths of white light. The remaining wavelengths remain and combine to give the interference color we see when we use crossed polarizers. Interference colors vary in hue with the retardation according to a characteristic sequence known as Newton's series (the same colors seen in soap bubbles and oil slicks). Newton's series is *not* a white light spectrum; it is divided into "orders," with the end of each order marked by a red-violet color representing one full wavelength retardation. The first order starts with black ($r = 0$), and for the first 250 nm or so the intensity of all wavelengths in white light is almost uniformly reinforced, resulting in hues of gray and white. This is followed by yellow and orange hues, and ends with the very important, intense, narrow "first order red" at about 550 nm. The second order has intense blue, green, yellow, orange, and finally red hues at about 1,100 nm. Successive orders become paler and paler; the fourth to tenth orders show only a diminishing pink and green. Above about tenth order we have only "white of high order." Detailed treatment of the mechanism of interference color production can be found in Gahm (2).

The thickness of a substance, such as a crystal or fiber, must be measured along the same direction the retardation is measured. This is fine for cylindrical fibers since the measured diameter is also the thickness. The proper thickness for crystals often can be measured by "crystal rolling." To do this, the particle must be mounted between slide and coverglass in a viscous liquid. Sliding the coverglass with a needle rolls the crystal to a position where the thickness can be measured directly with a

calibrated eyepiece micrometer. Some non-cylindrical fibers and polymer films can be cut with a razor at a precise 45-degree angle and the thickness measured as the horizontal projection of the cut. Finally, polymer films can be measured directly, using a thickness gauge before mounting.

Retardation all adds up

Retardation increases linearly with both the thickness of a substance and with the birefringence (Figure 5): the greater the thickness, the greater the retardation; the greater the difference between the refractive indices, the greater the retardation (or higher the interference color). That is,

$$r = t\,(n_2 - n_1)$$

where r is the retardation (interference color) expressed in nanometers (nm); t is the thickness, which must also be expressed in nanometers (multiply the reading in micrometers by 1000); and $(n_2 - n_1)$ is the numerical difference between the refractive indices (birefringence).

Michel-Lévy published the chart that bears his name in Paris in 1888 (3). The chart is based on the above equation which also can be written:

$$t = \frac{r}{n_2 - n_1} \text{ or } n^2 - n^1 = \frac{r}{t}$$

Hence, any one of these parameters can be determined from the other two.

Referring to the chart, we see the thickness, t, increases vertically along the ordinate on the left side. The path difference, or retardation, r, increases as we go right along the bottom of the chart; the names of the interference colors are also given. First-order red falls at about 550 nm; second-order red at twice 550, or 1100 nm; third-order red at 1650 nm, etc. The birefringence, $(n_2 - n_1)$, starts on the top of the chart at the left and proceeds all the way to the right and down the right side for increasing values. The diagonal lines represent the birefringence values listed at the upper end of each diagonal line. This is so because $t = r/(n_2 - n_1)$ is the equation for a straight line through the origin of the coordinates, with a slope of

$$\tan\theta = \frac{1}{n_2 - n_1}$$

Each line is assigned an angle θ, and, thereby, a special value $(n_2 - n_1)$. The names of numerous substances appear opposite their characteristic maximum birefringence value on some versions of the chart.

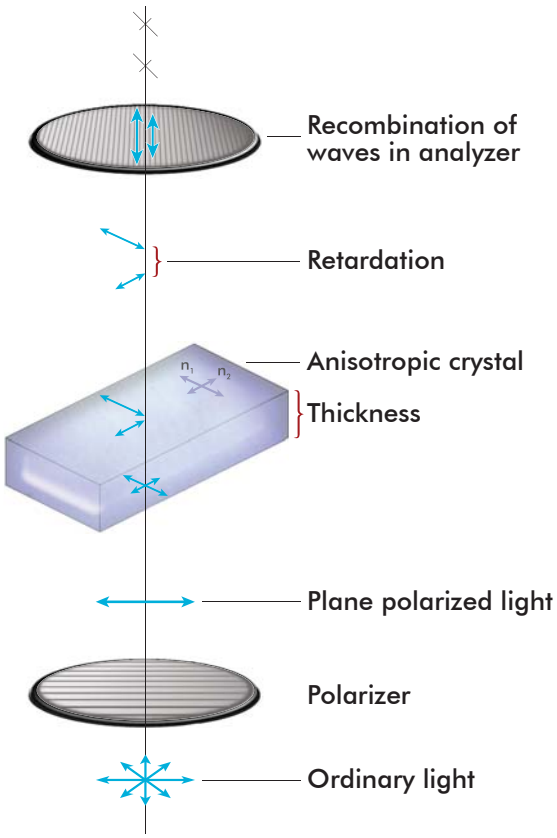

Figure 5

Retardation depends on crystal thickness, and numerical difference between the refractive indices n_1 and n_2.

Know two, find the other

Since the Michel-Lévy chart shows the interrelationships between thickness, birefringence, and interference color, the microscopist can determine any one of these from the chart if they know the other two. Several examples will illustrate this:

Suppose a cylindrical synthetic fiber 15 µm in diameter shows a maximum interference color corresponding to about 900 nm. This is determined by orienting the length of the fiber 45 degrees to the vibration directions of the crossed polarizers and comparing the color running down the *center* of the fiber to the colors in the chart. The order is found by noting the number of reds between the center and either edge of the fiber. One must be very careful here because the colors are very, very close together at the edge of a cylindrical fiber. It is often better to count orders on a taper-cut end of a fiber. In the present example, we pass through only first-order red, indicating the yellow at the center is second order. To determine the birefringence, we look for 900 nm along the bottom of the chart, and move vertically until we reach a horizontal line corresponding to a thickness of 15 µm on the ordinate. There will be a diagonal line where the two lines cross. We now follow the diagonal line to the upper right to read the birefringence, 0.060, at the top of the chart. Looking up this value in a table for birefringence of synthetic fibers we learn that a fiber having this birefringence is nylon. We could also have calculated birefringence from $(n_2 - n_1) = r/1000\ t$:

$$n^2 - n^1 = \frac{r}{1000\ (t)} = \frac{900}{1000\ (15)} = 0.06$$

Suppose now we wish to predict what interference colors would be observed on a sieved sample of the mineral wollastonite, randomly oriented, in which the maximum vertical dimension is 40 µm. The known birefringence of wollastonite from analytical tables is 0.014. On the chart, we look along the top until we come to 0.014, where we find a diagonal line. We follow this line to the lower left until it intersects a line corresponding to a thickness of 40 µm. At the point where these lines cross we find an interference color slightly more purple than first-order red. Thus, wollastonite particles 0 nm to 40 nm thick will show first-order interference colors of black, gray, white, yellow, orange, red, and purplish red, depending on their thickness and orientation. Again, one could solve this problem with the equation:

$$r = 1000\ (0 \text{ to } 40)\ X\ 0.014 = 0 \text{ nm to } 560 \text{ nm}$$

Finally, suppose we have a rock thin-section containing the mineral augite (birefringence 0.024) showing an optic normal interference figure and a first-order red interference color 550 nm. It is desired to know the thickness of the section. At the top of the chart we find the birefringence 0.024 and follow the diagonal line until it intersects the 550 nm line on the abscissa. From the coordinates we go directly left to the thickness on the ordinate and find 23 µm. Once again we can find the solution from the equation:

$$t = \frac{550}{1000\ (0.24)} = 23\ \mu m$$

Other orientations of augite would show lower order polarization colors.

Thus, the Michel-Lévy chart enables the microscopist to rapidly determine thickness, birefringence, or retardation, knowing the other two quantities.

A problem arises in using the Michel-Lévy interference colors chart when it comes to determining the order of a particular color, especially with thicker specimens of high birefringence or very thin specimens of low birefringence. Accessory *retardation plates* are interposed in the light path, usually in a slot between the microscope objective and the analyzer, to help determine the order of a particular color. These accessory plates may be made of mica, gypsum, quartz, or calcite. They help to determine the order by adding or subtracting fixed or variable amounts of known retardation to that shown by the specimen. Those with fixed amounts of retardation, such as the quarter-wave plate (~137 nm) and the first-order red plate (~550 nm), help determine the first order and, to some extent, the second. A wedge made of quartz may determine up to three, four, five or six orders. When we come to fractions of a wavelength, say λ/30, or many wavelengths, say 120λ, we need rotary accessory plates, such as the Ehringhaus and Brace-Köhler, but that is the subject of compensators.

The Michel-Lévy interference color chart is one that microscopists will find increasingly useful as they become more familiar with it. Its potential applications to the analysis of transparent particles are virtually unlimited. For a detailed history of this color chart, which has enabled polarized light microscopists to identify unknown particles for the last 125 years, see Delly (4).

Color clue to film thickness

Incidentally, a similar chart can be used to relate thin film colors to film thickness and refractive index (Figure 6). Newton's series of interference colors is displayed in the same color sequence by thin films of increasing thickness. The makes it possible to quickly estimate thickness of corrosion or contamination films. In the laboratory of McCrone Associates in Westmont, Illinois, interference films are constantly used to tell if electron microscope support films are thin enough. The polymer films, *e.g.,* collodion, used to cement small particles to beryllium plates for microprobe analysis interfere with the probe analysis if too thick; hence, must be very thin. The interference colors shown by such films help control this critical parameter.

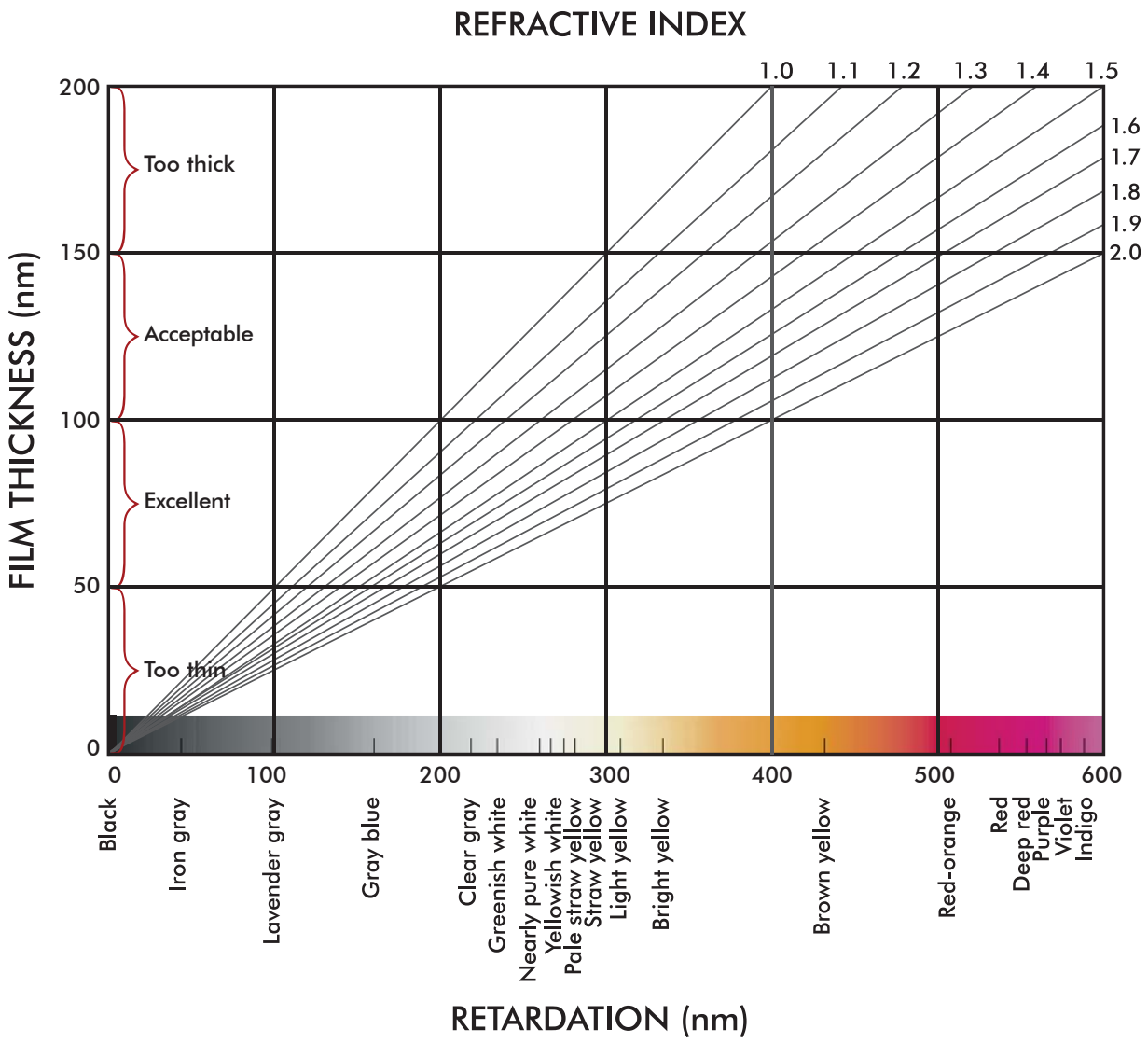

Figure 6

Relationship between thin film thickness, refractive index, and retardation.

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