A microscope able to provide simultaneous physical, chemical, and crystallographic data on particles down to 500 nm would surely be hailed as an incredible instrument. This microscope would likely find itself rapidly integrated into a variety of materials analysis procedures. One may be surprised to learn that a microscope with these capabilities has existed for more than 170 years. The polarized light microscope (PLM) has demonstrated its value as an indispensable analytical instrument with continuous usage and the development of new applications since its earliest reported use circa 1834. However, although the modern PLM is more useful than ever, there has been a general decrease in the awareness of its operation and capabilities in materials analysis. Several factors have contributed to this decrease, including 1) a sharp decline in optical crystallography course offerings with the retirement of key professors; 2) changes in academic and research funding trends; and 3) the popularity of other microscopes, namely the scanning electron microscope with energy dispersive spectroscopy (SEM-EDS); and the scanning probe microscope (SPM). Microscopists seeking to understand and apply the PLM must now rely on texts, on-the-job training, or training at the McCrone Research Institute (Chicago, IL). However, those microscopists who invest the time to become proficient with the PLM will enjoy the many advantages this technique offers in routine and specialized analyses. Those who choose to remain unaware of its capabilities are not fully appreciating the foundation on which so much of our current analytical knowledge is based.

Optical crystallography

Depending on the crystal system and the history (processing, growth, etc.), a material may possess one refractive index (i.e., n), two (n_ε, n_ω), or three (n_α, n_β, n_γ) (see Table 1). The six crystal systems are defined by the length (a, b, c) of each crystallographic axis (X, Y, Z) and their interaxial angles (α, β, γ). These crystallographic constants govern the symmetry of a material’s properties (Neumann’s principle). If the magnitude and orientation of the refractive index (or any other vector property) is expressed as vectors emanating from a common point, then the locus of all points will map a three-dimensional surface called an indicatrix (Figure 1). The shape of the indicatrix for the six crystal systems is therefore a sphere (isometric), a biaxial ellipsoid (hexagonal or tetragonal), or a triaxial ellipsoid (orthorhombic, monoclinic, or triclinic). By virtue of the lengths of the indicatrix axes, restrictions are placed on the possible cross-sectional shapes. Figure 1 shows the principal sections (i.e., an ellipse parallel to two of the principal refractive index values [n_ε, n_ω, n_α, n_β, n_γ]), circular sections, and random sections for the three indicatrix types. For the random uniaxial sections, either the major (uniaxial negative) or minor (uniaxial positive) axis will fit within an ellipse, whereas in the random biaxial sections, the surface will resemble an ellipsoid.
positive) radius has the principal value \( n_{\omega} \), the other radius has an intermediate (prime) length (i.e., \( n_{\epsilon'} \)). The random biaxial sections may be semirandom or random depending on whether one (semirandom) or both (random) of the ellipse axes are of a prime length (i.e., \( n_{\alpha'} \) or \( n_{\gamma'} \)). When performing PLM, crystals should be envisioned with their indicatrix orientation known. In doing so, the PLM provides a simple and direct means with which to rapidly evaluate the orientation and degree of anisotropy exhibited by a given molecu-
Polarized Light Microscopy

This provides data highly complementary in nature to other crystallographic techniques, e.g., single-crystal X-ray diffraction and electron backscatter diffraction (EBSD).

The simplest PLM is essentially a bright-field microscope with a rotating stage and plane-polarizing filters placed below (the polarizer) and above (the analyzer) the specimen. Figure 2 illustrates several important aspects concerning the interaction between the incident plane polarized light (PPL), the optically anisotropic sample, and the analyzer. By way of this simple arrangement, a crystal may be rotated so that its refractive indices and birefringence may be determined along with a host of other optical, physical, chemical, and crystallographic properties or qualities (Table 2).

In Figure 2, position 1, in-phase, east-west (E-W) plane polarized light enters an anisotropic material and is split into a slow ray (higher index) and fast ray (lower index) with their respective vibration directions parallel to the major and minor axes of the indicatrix's cross-section (see inset of indicatrix cross-section). The intensity of these rays is dependent on the angle between their vibration direction (that is, the major or minor axis) and the incident E-W polarized light. Consider a hypothetical PPL image containing the uniaxial positive \((n_e > n_o)\) crystals shown in Figure 3. In position 1, the crystal's minor axis \((n_o)\) is E-W (major axis must therefore be north-south [N-S]). Therefore, all of the incident light will be transmitted with its vibration direction parallel to this refractive index. If the stage is rotated 45° (position 2), then equal quantities of the incident E-W light will vibrate along \(n_o\) and \(n_e\). When the stage is rotated another 45° (position 3), the major axis \((n_e)\) is E-W, and the light will travel at a lesser velocity along this higher refractive index (i.e., slow) direction. The uniaxial crystal at location 4 is oriented with the \(n_o\) axis vertical; therefore, the indicatrix view presented is that of a

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**Table 2: Properties and Qualities Measured by PLM**

<table>
<thead>
<tr>
<th>Property</th>
<th>Definition/Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichroism, pleochroism</td>
<td>Preferential absorption of light along specific vibration directions within the crystal</td>
</tr>
<tr>
<td>Refractive index</td>
<td>Velocity of light in vacuum/velocity in material</td>
</tr>
<tr>
<td>Birefringence</td>
<td>Variation in refractive index ((n_{max} - n_{min})) (see Table 1)</td>
</tr>
<tr>
<td>Sign of elongation</td>
<td>Orientation of (n_{slow}) relative to crystal's length</td>
</tr>
<tr>
<td>Extinction type and quality</td>
<td>Provides direction of indicatrix axes relative to crystallographic axes</td>
</tr>
<tr>
<td>Interference figures</td>
<td>Crystal system determination</td>
</tr>
<tr>
<td>Optic axial angle (2V)</td>
<td>The angle between the normals to the two circular sections of a biaxial crystal</td>
</tr>
<tr>
<td>Optic sign</td>
<td>See Table 1</td>
</tr>
<tr>
<td>Dispersion</td>
<td>Variation of refractive index, birefringence, and optic axial angle “2V” with wavelength of incident light</td>
</tr>
</tbody>
</table>

Other properties/qualities:

1) Morphology/texture
   1) Crystal form, point group symmetry, cleavage, growth habit
2) Microstructure
   2) Grain orientation, size, and intergrowth
3) Purity
   3) Chemical and structural homogeneity
4) Color, size

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Figure 3: Hypothetical field of view in plane polarized light showing the shape of the indicatrix section for five uniaxial positive crystals. Crystals 1, 2, and 3 show the same principal section \((n_e - n_o)\) in different orientations relative to the E-W polarizer (see text). Crystals 2 and 5 show the angle of the two crystal vibration directions relative to the E-W polarizer. Crystal 2 is in the 45° position, which passes equal intensities of \(n_e\) and \(n_o\). Crystal 4 shows the circular (isotropic) section. Crystal 5 shows a random section with indices \(n_e\) and \(n_o\). In its current position, the E-W refractive index is slightly greater than \(n_o\).
The indicatrix for crystal 5 is oriented such that the section is elliptical, but the major axis ($n_{\epsilon}$) is less than $n_{\omega}$ in length. In this orientation, the $n_{\epsilon}$ index cannot be measured for any stage position.

Continuing along the ray path in Figure 2, by the time the fast ray (in red) reaches position 2, the slow ray (in blue) is about one wavelength behind, that is, the rays are separated by a path difference ($\Delta$) and may no longer be in phase. As each ray reaches the analyzer, only its N-S vibrating components may be transmitted. The interference of these slow and fast rays produces an interference color that can be related back to the path difference (also called retardation) by Eq. (1).

$$\Delta = \text{thickness} \times \text{birefringence}$$

When crystal 2 in Figure 3 is being viewed in cross-polarized light (i.e., analyzer inserted), it will show the brightest interference color because the largest portion of the $n_{\omega}$ and $n_{\epsilon}$ rays will pass the analyzer. Crystals 1 and 3 will appear black in XPL because the N-S analyzer will transmit none of the E-W vibrating light. Thus, with every 90° of stage rotation, a crystal will change from a position of maximum brightness to extinction. Note that the surrounding mounting media is isotropic (e.g., Cargille® liquid [Cargille Laboratories, Inc., Cedar Grove, NJ], water) and will remain dark under XPL during stage rotation, as would any isotropic material or any anisotropic material when viewed perpendicular to the circular section (e.g., crystal 4 in Figure 3). This is so because isotropic materials do not split the incident PPL into two mutually perpendicular, plane-polarized rays.

The interference color chart (Figure 4) shows a graphical solution to Eq. (1) over a range of typical values for birefringence, thickness, and retardation. This color chart serves as a standard for measuring

**Table 3 Classes of materials and industries that benefit from the PLM**

| Hazardous and regulated materials— asbestos, silica, white powders, ash, spores, pollen, mold |
| M inerals— metal ores (oxides, sulfides, silicates, zeolites, clays) |
| Synthetic polymers— PTFE, acrylic, nylon, polypropylene, polystyrene, etc. |
| Electronic and photonic materials— dielectrics, semiconductors, ceramics, composites, metals |
| Organic chemistry— pharmaceuticals, explosives, herbicides, dyes, adhesives, organometallics |
| Forensics— hair, drugs, poisons, munitions, paint, glass, soil, ink, currency, residues |
| Building materials— concrete, wood, asphalt, metal, paint, coatings |
| O ther materials— paper, textiles, food and feed, pigments, textiles, bone, fossil, gemstones, pottery |

Figure 4 Interference color chart showing the range of retardation colors from 0 to 1800 nm displayed by a birefringent crystal up to 50 µm thick. Note that the birefringence values wrap from the left y-axis to the top x-axis with example materials given.
(by eye) the retardation value so that the birefringence can be measured if the thickness is also measured or known. However, note that the measured birefringence will only be the true (i.e., maximal) birefringence if the crystal is oriented in the principal “n<sub>e</sub>-n<sub>ω</sub>” or “n<sub>γ</sub>-n<sub>α</sub>” section.

Taken collectively, all of the optical, crystallographic, and physical qualities observed for a sample (Table 2) are usually sufficient to identify a complete unknown or at least narrow its identity to just a few possibilities. With additional analyses, e.g., chemical analysis (EDS or microcrystal tests) or structural analysis (µFTIR, X-ray diffractometry [XRD], selected area electron diffraction [SA ED], or EBSD), it is seldom that a material can escape identification.

**Applications**

Optically anisotropic (i.e., birefringent) materials include 1) crystals belonging to the tetragonal, hexagonal, orthorhombic, monoclinic, and triclinic crystal systems; 2) crystals or amorphous substances that are strained during processing or service (e.g., tempered glass, many extruded or formed materials); 3) fibers and films that contain oriented polymers (e.g., nylon, hair, wood, acrylic, polypropylene); and 4) liquid crystals and optically active gases, liquids, and solutions. Materials possessing very low structural order may appear isotropic by eye, but may actually be very weakly birefringent when viewed using specialized accessories (e.g., liquid crystal polarizers, Brace-Kohler compensator). Isotropic materials include most liquids and gases, amorphous solids, and isometric crystals. Table 3 lists classes of materials and industries in which PLM has broad application.

Enough cannot be said for the PLM regarding its ability to present images in such stunning contrast that the microscopist always has instantaneous sight recognition for certain materials. Figure 5 shows the characteristic appearance of potato starch grains in XPL. Figure 6 depicts a side-by-side image through a comparison (forensic) microscope of a Caucasian hair and a rabbit hair. There is no mistaking these substances, and these are three materials from thousands that have an extremely characteristic set of optical properties and general appearance. Stereomicroscopes equipped with polarizers have great utility, especially for tasks requiring low magnification and high contrast, such as surveying heterogeneous samples (e.g., filters, tape lift evidence), sample preparation, and photodocumentation in support of other types of microanalysis.

Polarized light is a requisite for many other optical contrast techniques such as differential interference contrast, Kerr contrast, and Hoffman modulation contrast, and can increase the amount of data obtained with other techniques such as darkfield and dispersion staining. Figure 7 shows the same field of view of a polished α-Fe<sub>2</sub>O<sub>3</sub> sample using PPL (a), XPL (b), and DIC (c). The PPL image shows the general luster and pitting, the XPL image clearly shows the crystal boundaries and their interference color, and the DIC image shows the microtopographic features in high contrast. The PLM can also provide visual support for microchemical tests for specific elements, valence states, anionic groups, proteins, fats, etc.

The PLM is not only useful for making static observations of optical properties. Homemade and commercially available accessories can also adapt the PLM for observing dynamic processing and performing additional measurements.
This short list of applications is meant to stress the many capabilities of the PLM and the light microscope in general. Each type of microscope (light, electron, scanning probe, etc.) was designed with specific applications in mind and should be used with knowledge of their strengths and weaknesses. While there are overlaps in applications of the various types of microscopes, each type is superior in its own way. The use of the polarized light microscope does not need to be defended; its capabilities speak for itself. PLM only needs to be promoted.

References

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Figure 7 Reflected light images of $\alpha$-Fe$_2$O$_3$ using a) PPL, b) XPL, and c) differential interference contrast (DIC).

For example, hot/cold stages, high-pressure diamond-anvil cells, environmental chambers, and applied electric fields allow one to observe/measure processes such as softening/melting point, polymorphic transformations, volume expansion, ferroelectric domain structure, heavy metal diffusion into zeolites, bacteria respiring on mineral surfaces, hydration/dehydration behavior, and crystal growth/etching mechanisms.