OSAC PROPOSED STANDARD 2025-S-0011
Standard Guide for Polarized Light Microscopy in the Forensic Examination and Comparison of Soils

Trace Materials Subcommittee
Trace Evidence Scientific Area Committee (SAC)
Organization of Scientific Area Committees (OSAC) for Forensic Science

OSAC Proposed Standard

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Standard Guide for Polarized Light Microscopy in the Forensic Examination and Comparison of Soils

1. Scope

1.1 This guide covers the use of polarized light microscopy (PLM) for the identification and comparison of the mineralogical components of soils (to include unconsolidated geological materials) for forensic applications.

1.2 Soils are often complex mixtures of a variety of components. This guide is tailored to the microscopical examination and comparison of the geological components of soils in grain mounts.

1.3 This standard is intended for use by competent forensic science practitioners with the requisite formal education, discipline-specific training (refer to Practice E2917), and demonstrated proficiency to perform forensic casework.

1.4 The values stated in SI units are to be regarded as standard.

1.5 This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.

1.6 This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

2. Referenced Documents

2.1 *ASTM Standards*:

E620 *Practice for Reporting Opinions of Scientific or Technical Experts*
E1492 *Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Science Laboratory*
E1732 *Terminology Relating to Forensic Science*
E2228 *Guide for Microscopical Examination of Textile Fibers*
E2917 *Practice for Forensic Science Practitioner Training, Continuing Education, and Professional Development Programs*
E3254 *Practice for Use of Color in the Visual Examination and Forensic Comparison of Soil Samples*
E3272 *Guide for Collection of Soils and Other Geological Evidence for Criminal Forensic Applications*
E3294 *Guide for Forensic Analysis of Geological Materials by Powder X-Ray Diffraction*
E3423 *Guide for Forensic Analysis of Explosives by Polarized Light Microscopy*
C295/C295M *Guide for Petrographic Examination of Aggregates for Concrete*

2.2 *ISO Standards*

ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories

3. Terminology

3.1 Definitions – For definitions of terms used in this practice relating to forensic science, refer to E1732.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *alpha* (α)—symbol representing the lowest of the three principal refractive indices of a biaxial crystal (E3423).

3.2.2 *anisotropic*, n — a characteristic of an object in which the refractive index differs depending on the direction of propagation or vibration of light through the object (E2228).

3.2.3 *anomalous interference colors*, *n* – colors produced when birefringence and retardation colors are significantly different for different wavelengths of light (**1**).

3.2.4 *Becke line*, *n* – a bright halo near the boundary of a transparent material that is mounted in a medium that differs from its refractive index.

3.2.5 *Becke line method*, *n* – a method for determining the refractive index of a material relative to its mounting medium by noting the direction in which the Becke line moves when the focus is changed.

3.2.6 *beta* (β) — symbol representing the intermediate principal refractive index of a biaxial crystal (E3423).

3.2.7 *biaxial*, adj — an anisotropic crystal in the orthorhombic, monoclinic, or triclinic system with three principal refractive index directions (α, β, γ) and two optic axes that are isotropic (E3423).

3.2.8 *birefringence*, *n* – the numerical difference between the maximum and minimum refractive indices of anisotropic substances.

3.2.8.1 Discussion: the maximum birefringence is the difference between alpha and gamma (biaxial) or epsilon and omega (uniaxial).

3.2.9 *compensator*, *n* – a device that can be inserted into the optical path of a polarized light microscope to introduce a fixed or variable retardation.

3.2.10 *conoscopic examination*, *n* – visualization of the back focal plane of the objective, often used to study the illumination setup of the microscope or an interference figure of the specimen being examined.

3.2.11 *epsilon* (ε) —any vibration direction in the plane of the c axis for uniaxial crystals (E3423).

3.2.12 *extinction*, *n* – the condition in which a birefringent particle appears dark when viewed between crossed polarizers.

3.2.13 *gamma (γ)* —symbol representing the highest principal refractive index of a biaxial crystal (E3423).

3.2.14 *grain type*, *n* - a categorical label that identifies particle(s) that are defined by a consistent set of parameters (e.g., morphological, descriptive, chemical), that differentiates them from the larger population of grains in the soil.

3.2.15 *interference colors*, *n* – colors produced by the combined interference of out-of-phase rays of white light when a birefringent material is observed at a non-extinction position between crossed polarizers.

3.2.15.1 *Discussion* – Also known as retardation colors, typically reported in nanometers, or order.

3.2.16 *interference figure*, *n* – pattern observed during conoscopic examination of an anisotropic material that consists of a combination of extinction positions and interference colors corresponding to the full cone of directions by which the sample is illuminated (**3,** E3423).

3.2.17 *isotropic*, *adj* – a characteristic of an object in which the refractive index remains constant irrespective of the direction of propagation or vibration of the light through the object (E2228).

3.2.18 *mineral, n*—a naturally occurring inorganic element or compound having an orderly internal structure and characteristic chemical composition, crystal form(s), and physical properties, or an element or chemical compound that is crystalline and that has formed as a result of geological or pedogenic (soil-formed) processes (E3294).

3.2.18.1 *Discussion*—Artificial and biogenic crystalline materials are not minerals but can occur in geological materials (for example, cement powder, lime, lye, biogenic calcite, biogenic hydroxyapatite, bricks) and can be characterized by PLM.

3.2.19 *omega* (ω)—any vibration direction in the plane of the a axis for uniaxial crystals (E3423).

3.2.20 *optic axial angle (2V)*, n—the acute angle between two optic axes of a biaxial crystal (E3423).

3.2.21 *optical indicatrix*, *n* – a theoretical three-dimensional construction that shows the relationship between vibration direction of light and the refractive index for solids.

3.2.22 *optic sign, n* – determined by the relationship of the refractive indices of a material; for uniaxial crystals: if $\varepsilon > \omega$, the optic sign is positive (+); if $\omega > \varepsilon$, the optic sign is negative (-); for biaxial crystals: if $\gamma-\beta > \beta-\alpha$, the optic sign is positive (+); if $\gamma-\beta < \beta-\alpha$, the optic sign is negative (-).

3.2.23 *pleochroism*, *n* – the property of exhibiting different colors when viewed along different axes of a material relative to the polarization plane of the light used to illuminate the sample (E2228).

3.2.24 *polarized light microscope*, *n* – a compound microscope equipped with two polarizing filters, one below the stage (the polarizer) and one above the stage (the analyzer) (E2228).

3.2.25 *refractive index (RI)*, *n* – the ratio of the velocity of light in a vacuum to the velocity of light in some medium (E2228).

3.2.26 *relative refractive index*, *n* – the estimate of the difference in the refractive index of a material in relation to the refractive index of its surrounding medium, often established via the Becke line method (E2228).

3.2.27 *relief, n* - contrast between a particle or crystal and its media due to the difference between their refractive indices. The greater the numerical difference in refractive indices, the greater the relief (E3423).

3.2.28 *retardation*, *n* – the actual distance of one of the doubly refracted rays behind the other as they emerge from an anisotropic material; dependent upon the difference in the two refractive indices and the thickness of the material.

3.2.29 *sign of elongation*, *n* – relationship between the orientation of the vibration directions of the slow and fast rays in an elongated, anisotropic substance.

3.2.29.1 *Discussion* – when the long axis - is aligned with the high index direction (slow ray) the sign of elongation is positive (+) (also called length slow), when the long axis is aligned with the low- index direction (fast ray) the sign of elongation is negative (-) (also called length fast); should not be confused for optic sign.

3.2.30 *twinning*, *n* – an intergrowth of two or more crystals within a single grain that bears a definite angular relationship and coincides with a potential crystallographic face; randomly intergrown crystals are not twins.

3.2.31 *uniaxial*, adj—an anisotropic crystal in the tetragonal or hexagonal system having one optic axis (isotropic direction) and either two (tetragonal) or three (hexagonal) directions which are alike and perpendicular to the direction of the optic axis (E3423).

4. Summary of Guide

4.1 This guide describes the use of PLM for characterization of unconsolidated geological materials examined as trace evidence. The guide recommends common sample preparation procedures to enable examination of grain mounts and describes the use of optical properties and morphological characteristics to identify and describe grain assemblages.

5. Significance and Use

5.1 There are three main goals of forensic soil examinations: (1) identification of an unknown material as soil or sediment, (2) comparison of two or more soils to assess if they could have originated from a common source or to exclude a common source based on the observation of exclusionary differences, and (3) characterization of soils to restrict their potential geographic origins as part of a provenance investigation. Characterization of the unconsolidated geological materials within soils using PLM can assist with addressing these analytical goals.

5.2 Microscopical examination is non-destructive to geological materials and can be implemented at any point in an analytical scheme, although such examinations often require some level of sample preparation.

5.3 PLM provides a means for characterizing and identifying individual particles from unconsolidated geological materials, based on their morphology and optical properties. The identified components can be compared between samples or, when applicable, to published data for interpretation or geographic attribution purposes.

5.4 This guide is intended to be used with other methods of analysis (e.g., palynology, color determination, X-ray diffraction (XRD), or scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDS)) within a more comprehensive scheme for the forensic analysis or comparison of soils (**4**, **5**, **6**).

5.5 This guide does not address the other commonly encountered soil components (e.g., clays, plant tissues, pollen, spores, humic matter, *etc.*). Certain anthropogenic materials commonly found in soils can be identified and compared following the methods outlined in this guide.

5.6 This guide does not discuss the examination of rocks or building materials (e.g., bricks and concrete, *etc.*) by polarized light microscopy (C295/C295M).

5.7 Limitations:

5.7.1 The capacity to identify crystalline grains by PLM depends on the following: the specific mineral (some have more diagnostic optical or morphological characteristics); the condition, amount, and size of the grains.

5.7.2 The identification of minerals might require confirmation with orthogonal techniques.

5.7.3 The spatial heterogeneity of soils is location-specific and should be considered in the interpretation of soil comparisons and provenance examinations (see E3272).

6. Materials

6.1 *Polarized light microscope* - A polarized light microscope, equipped with two linear polarized filters, a rotating stage, substage condenser, and a compensator slot, are needed for the examination of geological materials. The following accessories are highly recommended:

6.1.1 Bertrand lens - used for conoscopic examinations.

6.1.1.1 An alternative to using a Bertrand lens for conoscopic examination is to directly observe the back focal plane of the objective by removing the eyepiece; however, the image will appear smaller. A phase-centering telescope can also be used to observe the back focal plane of the objective for conoscopic observation.

6.1.2 Objectives - Strain-free objectives with a range of magnifications (approximately 5x - 60x magnification). A high magnification objective with large numerical aperture (e.g., 40x with 0.65 or greater numerical aperture) is recommended for conoscopic viewing.

6.1.2.1 A dispersion staining objective can aid in mineral identification.

6.1.3 Mechanical stage (or attachment) - for grain counting and systematic observation.

6.1.4 Transmitted (required) and oblique reflected (recommended) illumination sources.

6.1.5 Phototube and camera for documentation.

6.1.6 Compensators, at least one among the following:

6.1.6.1 Fixed retardation compensators: typically quarter-wave (137 nm), or full-wave (530 to 550 nm) plates.

6.1.6.2 Variable compensators: e.g., quartz wedge, Berek compensator.

6.1.7 Immersion oil, if the optical component is designed for use with oil.

6.2 *Reference Materials*

6.2.1 Known mineral specimens/grains

6.2.2 Mineral reference data - reference data of morphology and optical properties for known minerals are available in various atlases and texts (**7**, **8**, **9**, **10**, **11**, **1**).

6.3 Sample preparation materials - A range of common laboratory materials can be helpful in preparing grains for microscopical examination. Suggested materials include:

6.3.1 Metal sieves or disposable sieve cloth and holders with mesh sized in the range of 50 µm to 250 µm.

6.3.1.1 Disposable sieve cloth reduces the chance of cross-contamination.

6.3.2 Containers for washing grains in aqueous solutions.

6.3.3 Surfactants for washing grains, commonly sodium hexametaphosphate, dilute alcohol, or ultrasonic cleaning detergent.

6.3.4 Ultrasonic bath.

6.4 *Mounting Materials*

6.4.1 Glass slides and coverslips

6.4.2 *Mounting media* – Mounting media with known indices of refraction (commonly 1.540 and 1.660) are recommended (refer to §7.4). Specific RI values are not typically required for grain identification. Relative refractive index data (refer to §8.3.4) are often sufficient for mineral identification; thus, an entire series of RI liquids is typically unnecessary.

6.4.3 Helpful tools for mounting include tungsten needles, handheld magnet, and forceps.

7. Specimen Preparation

7.1 The amount of sample preparation required for microscopical examination of soils will depend on the condition of the sample and goal of the analysis. When comparing samples, specimens should be prepared in the same manner.

7.2 The processing of soils can include grain washing, particle size fractionation, density, and magnetic susceptibility separations. Procedures for such separations related to forensic soil examinations are detailed in published references (**12**, **7**). In most cases, some sample processing is required.

7.2.1 Some geological materials can benefit from the removal of mineral grain coatings for examination by PLM (**13**).

7.3 The particle size ranges in grain mounts commonly examined by PLM are the fine (125-250 µm) and very fine sand grains (63-125 µm). Grains finer than this size range can be too small to permit measurement of their optical crystallographic properties while coarser grains are often polymineralic rock fragments and are too large for examination by PLM in grain mounts due to working distance and field of view limitations. Grain size separation can be achieved with clean, laboratory-standard metal sieves or disposable plastic sieve cloth. Alternatively, grain size separation by Stokes' settling law can separate grain sizes.

7.3.1 The USDA (**37**) defines fine sand and very fine sand slightly differently than is used in this practice (100 to 250 µm and 50 to 100 µm, respectively). This distinction is seldom important for forensic soil examination by PLM, but reports should note the size fractions analyzed.

7.4 *Mounting Considerations* - The following are suggested as a general guide to mounting specimens for examination by PLM.

7.4.1 Temporary mounting media, such as RI oils, allow for mineral grains to be easily recovered from microscope slide preparations and are preferred when subsequent instrumental analysis might be pursued. In addition, the optical orientation of particles mounted in RI liquids can be adjusted (rolled) to allow for different optical crystallographic properties (e.g., RI, orientation of the optical indicatrix) to be observed. Preparations utilizing permanent mounting media (with known refractive indices) (e.g., epoxy, UV-cured polymer, thermoplastics) are more easily shipped or archived and are recommended for reference collections.

7.4.2 Light (low density) mineral grains (commonly considered to be minerals with $\rho < 2.89$ to 2.96 g/mL) are typically mounted in 1.54 index of refraction media and heavy (high density) mineral grains (commonly considered to be $\rho \geq 2.89$ to 2.96 g/mL) in 1.66 index of refraction media. These indices of refraction are recommended since they are near the refractive indices of many of the commonly encountered minerals and provide adequate relief for evaluating their optical crystallographic properties (**12**, **3**). When necessary, media with different indices of refraction can be used (e.g., to increase contrast and enable surface texture observations or to gain additional relative refractive index information).

7.4.3 Soil samples that are not separated into light and heavy mineral fractions (e.g., due to sample size limitations) are often mounted in 1.54 index of refraction media. However, when necessary, the sample can be recovered from the temporary mounting media, washed, and remounted in a medium of another refractive index.

8. Examination with the Polarized Light Microscope

8.1 The optical crystallographic (§8.3) and morphological (§8.4) properties determined by PLM are employed in qualitative mineralogical identification. PLM can also be used to determine grain morphological features related to the parent material or transport and weathering history of the grains.

8.2 A microscope setup for Köhler Illumination is recommended for the forensic examination of soils (**3**). For comparisons, similar illumination and magnification range should be used.

8.3 Optical Crystallographic Properties

8.3.1 Numerous texts review the theory and application of optical mineralogy for the differentiation and identification of minerals (**7**, **8**, **9**, **10**, **11**, among others). Many of the key optical properties for mineral identification are summarized in §8.3.2 through §8.3.7.

8.3.2 *Color and Pleochroism* – To observe the color of grains and any inclusions or coatings, use a combination of transmitted and reflected illuminations. To determine if grains and inclusions exhibit pleochroism, plane-polarized light should be used while rotating the stage (Figure 1).

8.3.3 Reflected light microscopy is well-suited to the examination of mineral surface coatings and opaque minerals. The observation of various properties (color, reflectivity, pleochroism, bireflectance, etc.) as well as elemental analysis and

Raman spectroscopy on polished specimens are used to identify opaque minerals (14,15).

8.3.4 *Relative Refractive Index* – An evaluation of a particle's relative refractive index involves determining whether an immersed particle has a higher or lower RI relative to the surrounding medium, using the Becke line method (§3.2.3), oblique illumination, or dispersion staining (§8.3.4.3). An estimate of an approximate RI based on the observed relief and Becke line color(s) at grain edges can be made (9). Examples of high and low relief are shown in Figure 2. Uniaxial and biaxial minerals have more than one principal RI. To assess these RI values, the relative refractive indices for individual minerals should be determined at extinction positions.

NOTE 1: For uniaxial substances the principal refractive indices are labeled ε and ω, for biaxial crystals the principal refractive indices are labeled α, β, and γ. Between the principal refractive indices, materials exhibit intermediate refractive indices labeled ε' for uniaxial crystals and α' and γ' for biaxial crystals.

8.3.4.1 *Dispersion colors* – Colored Becke line fringes appear surrounding a particle that is immersed in a medium that has a RI close to the particle. The colors and directions of movement when the focus is changed can provide insight into the difference in RI between the particle and the mounting medium (9).

8.3.4.2 The consistent use of mounting media of a single known RI(standardized to a given temperature and wavelength) allows the observed relief and dispersion colors to be used in characterizing minerals (9). When a particle and the mounting medium share a common RI at specific visible wavelength, then dispersion colors are typically visible without the aid of a specialized dispersion staining objective; examples are shown in Figure 3 and described in (9).

8.3.4.3 *Dispersion staining* – The use of a dispersion staining objective enables rapid determination of the relative refractive indices of particles (16). This technique is commonly used for the identification of varieties of asbestos and other minerals (17, 18, 19).

8.3.5 *Interference Colors* – Grains possessing more than one RI generally will show interference colors between crossed polarizers when not aligned in an extinction position (Figure 4). The interference colors displayed by a mineral can be used to estimate its retardation. This value, along with an approximation of the thickness of the grain, can be used to estimate the birefringence of anisotropic materials, and thus, can be useful for identifying certain minerals. Isotropic materials have only one principal RI and do not yield interference colors (however, it is possible to have low-level strain birefringence in otherwise isotropic materials). Some minerals exhibit anomalous interference colors (e.g., zoisite/clinozoisite or titanite); the presence of anomalous interference colors is a useful characteristic for identifying certain minerals.

8.3.5.1 Dark-colored grains can obscure the observed interference colors.

8.3.6 *Extinction (Type and Character)* – The type of extinction can be categorized as parallel, symmetric, or oblique and the extinction character can be complete or incomplete. For an accurate assessment of extinction type, the crystallographic

orientation of the mineral must be determined (e.g., conoscopy or crystal morphology). The measurement of extinction angle relative to a mineral's elongation direction, cleavage, or twinning planes can sometimes be helpful to characterize/distinguish certain minerals (e.g., pyroxenes and amphiboles). Twinning (e.g., in feldspars and calcite) and strain (e.g., in quartz) can be observed through an examination of extinction. Examples of two types of extinction character are shown in Figure 5.

8.3.7 *Interference Figure and Optic sign* – Conoscopic examination of an anisotropic mineral grain is used to observe its interference figure. The interference figure can be used to classify the mineral as uniaxial or biaxial. The optic sign of a mineral grain can be determined by inserting a compensator or quartz wedge into the optical path (**10**). In addition, the 2V angle and dispersion of the optic axes of biaxial minerals can also be determined by examining the interference figure. The measured or estimated 2V angle can be used for mineral identification and for differentiation between members of the same mineral group.

8.3.7.1 While not typically necessary for qualitative mineral identification, the interference figure orientation can be used to locate appropriately-oriented mineral grains, thus permitting measurement of extinction angles, observation of principal refractive indices, and other crystallographic properties.

8.3.8 *Sign of elongation* – The sign of elongation can be determined for elongated grains and aid in mineral identification. This property typically relies upon the use of a compensator or quartz wedge and is categorized as positive or negative; it should not be confused with optic sign (§8.3.7)

8.4 Morphological Characteristics

8.4.1 *Shape and Cleavage* – The microscopic grain morphology can be described on the basis of properties including the presence of crystal faces (euhedral, subhedral, anhedral), roundness (rounded, sub-rounded, sub-angular, angular), and habit (equant, bladed, elongate, platy). These schemes are well described in published references (**20**). Additional morphological characteristics include twinning, cleavage, and fracture. For minerals that display euhedral to subhedral morphology, the crystal form(s) and habit can be assessed.

8.4.1.1 Automated grain morphometry can be used to quantify grain shapes (e.g., **21**).

8.4.2 *Inclusions and Zoning* – Internal structures within a single mineral grain represent additional characteristics useful in the comparison of multiple samples or the description of potential geographic origins. Inclusions may comprise fluid/gas bubbles or separate crystals; zoning should not be confused with twinning (refer to §3.2.23 and Figure 6).

8.4.3 *Surface Texture/Morphology* – Features on the surfaces of grains can provide information that often relates to its geological history (e.g., transport mechanism and local environment). Grain surface texture can provide detailed characterization of a mineral species, additional discrimination in the comparison of multiple samples, or assist in describing the local environment from which the grain originated; two examples of surface features are provided

in Figure 7. In addition, individual mineral grains can have surface coatings (e.g., iron oxides or clays) useful for provenance and soil comparison.

8.4.3.1 *Staining* – The visualization of surface features on mineral grains can be improved with the use of dyes to enhance surface texture(s) (e.g., quartz-silica using methylene blue) and coatings (e.g., clay mineral coatings using Malachite green in nitrobenzene).

8.4.4 Some organisms produce mineralized structures that are often observed in the fine to very fine sand-sized mineral fractions of soils (e.g., foraminifera, diatoms, phytoliths, or dinocysts). These can be fossilized or modern and their presence can be valuable for both comparisons and provenance interpretations.

9. Results

9.1 Identification/Classification

9.1.1 Evaluation of the numerous optical properties and morphological characteristics can enable grain identification to the mineral group (garnet group, feldspar group, etc.) and species levels (quartz, calcite, zircon, etc.). Measurements of optical properties are rarely required for qualitative mineral identification, although there are exceptions (e.g., plagioclase feldspars and members of the amphibole group) (see **8**).

9.1.1.1 A prepared table to record the optical properties of observed minerals can be helpful while characterizing a grain mount (for an example table, see **9**).

9.1.2 The characteristics needed for the identification of minerals are specific to the mineral group/species. Summaries of the optical properties for identification and differentiation of common soil mineral grains can be found in (**22**, **13**, **23**, **24**, **7**).

9.1.3 If a mineral identification to species or group is not made, grains can be described and categorized based on the observed microscopical characteristics. Further analytical characterization of the grain(s) should be considered if it would benefit the examination.

9.1.4 Biominerals (e.g., foraminifera) can be recognized based on their morphology and optical properties; their taxonomic identification requires specialized references or education (**25**).

9.2 Comparison between samples

9.2.1 *Grain types* – Identify or categorize grain types as minerals, mineral groups, lithic types, or morphotypes in samples to be compared by microscopical examination.

9.2.2 Compare notable morphological features, particularly grain shape, surface textures, and inclusions.

9.2.3 *Relative abundances* – Comparing the grain types and their abundances between samples can be meaningful in the forensic examination of soils. Two methods for the comparison of sample grain-type abundances include visual estimation (qualitative) and grain counting (quantitative).

9.2.3.1 *Visual Estimation* – Qualitative descriptors of abundance (major, minor, trace) can be assigned to the minerals/groups identified in a sample based on a visual assessment of the sample. A common scheme

for these descriptors is: major (~>10%), minor (~10%-1%), and trace (~<1%).

9.2.3.2 *Grain counting* – The purpose of grain counting in forensic mineralogical examinations is to quantify the components of a soil (**26**). In this procedure, mineral grain species are identified and tallied as the specimen is moved in uniform increments on a microscope stage. There are two principal methods for grain counting: line and field counting (**27**). Three hundred grains are typically sufficient for most routine examinations. After counting at least 300 grains, scan the grain mount for additional trace components that were not detected in the initial count (**13**).

NOTE 2 – The standard of counting at least 300 grains originated from a paper by Dryden (**28**). Trace constituents can be missed in this grain counting, so scanning all slides for all of their constituent components is recommended.

NOTE 3 – A prepared table of common mineral varieties can be helpful while characterizing a grain mount to document their presence or to tally grain counts (for an example table, see **24**).

9.3 Comparison to published data

9.3.1 The geographic location of unknown samples can be constrained by the comparison of their components or grain morphology to published data; e.g., identifying the location from which soil on a vehicle could have originated by comparison to geologic maps. For many localities, reliable mineralogical data are available (e.g., geological maps or USDA soil survey laboratory database-**29**).

9.3.2 Published mineralogical data can also be used to assess the rarity of a mineral at a known location (**30**).

10. Interpretation

10.1 Identification/Classification

10.1.1 Accuracy and specificity of grain assignments by PLM are dependent upon the quality of the reference materials, and the nature of the sample being examined.

10.1.2 *Mineral identification*

10.1.2.1 Mineral identifications can be made to different degrees of taxonomic specificity (e.g., mineral group, mineral species, or mineral varietal) depending on the quality of the reference materials (publications and physical reference collections), and the characteristics exhibited by the grains being examined.

10.1.2.2 Some minerals can be specifically identified by PLM, while other minerals can only be identified provisionally and require data from orthogonal techniques (e.g., elemental analysis, Raman spectroscopy, or XRD) to confirm identifications.

10.1.2.3 Mineralogy reference publications provide guidance on which minerals can be identified with high confidence by PLM. These references also address which minerals can be confused for each other by PLM and provide guidance on how to distinguish them (**10**, **7**, **13**). The ability to

distinguish optically similar minerals is dependent on their condition (e.g., weathering, coatings, or size) of the grain type(s) in question.

10.1.2.4 Grain types that cannot be definitively identified can still be useful for interpretation purposes. Unassigned grain types should be characterized and described (morphology, optical properties), documented, and considered during forensic comparisons. Orthogonal tests (refer to list in 10.2.2) might be able to assist in the identification of these phases or to further characterize them.

10.1.2.5 Accuracy of mineral identification - The interlaboratory study of Dunkl et al. (**31**) compared the accuracy of heavy mineral quantification by four methods, including PLM. Overall, PLM did not perform as well as the other three methods evaluated, but experienced participants in this study obtained results comparable to instrumental methods.

10.1.3 *Identification/classification of other grain types*

10.1.3.1 *Lithic types* — Polycrystalline lithic grains can be difficult to identify by PLM but can be categorized. Such particles can be further analyzed by making thin-sections and characterized by PLM or other instrumental methods (e.g., SEM-EDS, Raman spectroscopy).

10.1.3.2 *Biogenic particles* – The identification of soil particles as biological components/biominerals (e.g., foraminifera, shell fragments) by morphology is routine; taxonomic identification requires specialized references. The level of taxonomic specificity varies among taxa and the specimen condition (**25**).

10.1.3.3 *Anthropogenic particles* — The level of characterization of anthropogenic particles by PLM alone typically consists of identification of the particle type (e.g., paint, rubber, glass, or plastic) and gross morphological descriptors (color, shape).

10.2 Comparison

10.2.1 *Purpose of a comparison* — The goal of a microscopical comparison of soils is to decide if two samples could share a common source based on having similar properties or if there are exclusionary differences and, therefore, derived from different sources.

10.2.2 PLM is one part of a multi-analytical approach used in soil comparisons. Analysis of soil samples by orthogonal methods (e.g., grain coatings, SEM-EDS, palynology, color, or XRD) strengthens a comparison; however, when PLM alone clearly demonstrates variations in the components identified, their relative abundances, or differences in grain morphology between the samples, these results can provide sufficient evidence of an exclusionary difference.

10.2.3 The identification of rare minerals or anthropogenic particles by PLM increases the probative value of the evidence in a comparison. When available, mineral occurrence data can be used to substantiate the rarity of a mineral in general, or specifically within an area of interest.

10.2.4 *Similar samples* – The observation that multiple soil samples contain the same minerals or components with similar modal abundances and morphological characteristics supports the interpretation that the samples could have originated from a common source.

10.2.4.1 There could exist additional sources of soils that contain similar properties (modal abundance of mineral types and varieties).

10.2.4.2 The spatial extent of soils which share the same PLM characteristics can be constrained with sufficient known exemplars (E3272).

10.2.4.3 The compared samples could contain exclusionary differences that are not detectable by PLM (e.g., different populations of opaque minerals, different clay-sized minerals, color, or biological components). Orthogonal methods (e.g., palynology, color-E3254, XRD-E3294, Raman, or elemental analysis) can assist in detecting possible exclusionary differences.

10.2.5 *Samples with differences* — Differences can exist between the grain mounts of geological material that originated from the same source. Examples of explainable sources of variation include fractionation derived in the transfer and persistence of particles (**32**); contamination/alteration of one of the samples (e.g., by fire, stomach acid, or mixing); sample size limitations; or the representativeness of the known exemplars. Components present in trace abundances might not occur in all soils from the sample location (**30**). The absence of a component at a trace level by itself does not constitute an exclusionary difference.

10.2.5.1 If reasonable explanations exist for the observed differences, then justification for the differences should be documented and the difference is not considered exclusionary. For these cases, use additional orthogonal methods (e.g., palynology, SEM-EDS, or XRD) to further evaluate for exclusionary differences between the two samples.

10.2.5.2 If the differences observed cannot be explained, then they are considered to be exclusionary differences, and the soils are likely derived from different sources. Exclusionary differences could include variations in the modal abundance or morphology of grain-types, or the presence or absence of components.

10.2.6 *Insufficient for analysis* - Samples that are too small, mixed, adulterated, or not representative of their source do not lend themselves to comparison by PLM.

10.3 Sourcing (*syn:* geographic attribution or provenance)

10.3.1 PLM provides a means of mineral identification and morphological characterization that can be used, along with other observations, to aid in geographic attribution of soils, as the characteristics of grains (especially quartz grain surfaces) can be attributed to broad environments (e.g., sands from rivers, beaches, dunes, or glacial locations have specific characteristics) (e.g., **33**, **34**, **35** on geographic attribution in general and **36** on the origin of Fusen Bakuden, Japanese balloon bombs). Grain shapes and mineral varietal types can provide insights into the depositional setting and transport history of sediments, the geologic history of the grain or parent rock, weathering history, etc. The interpretation of PLM-derived results for provenance is highly case-specific.

10.3.2 PLM-derived mineral identifications and morphological observations can be compared to reference data and maps. The means for conducting such a comparison are beyond the scope of this document.

10.3.3 Biomineral grains (e.g., foraminifera) can be recognized by PLM and can be valuable for provenance interpretations. Modern biominerals can provide insights into the environmental/ecological characteristics of the source area. Microfossils can provide biostratigraphic constraints on the source of the sample when referred to an expert or taxonomic guide. Many other non-mineral soil components (anthropogenic materials, botanical fragments, etc.) can be recognized by PLM and are potentially informative during provenance interpretations.

10.3.4 PLM is also useful for recognizing and selecting grain types that can be used for specialized additional testing that is informative for provenance investigations (e.g., selecting mineral grains for geochronology).

11. Documentation

11.1 Refer to Practices E1492 and E620, and ISO 17025 for general guidance on reporting and documentation.

11.2 Document sufficiently to allow a second analyst to understand and evaluate all the work performed and independently interpret the data.

11.3 Documentation of geological materials examinations by PLM should include:

11.3.1 Any specimen preparation procedures performed (e.g., sieving, washing, density separation, mounting media).

11.3.2 The examiner's analytical notes provide a listing of the minerals, mineral varieties, and other components (characterized by their optical crystallographic and morphological properties) that are relevant for interpretation purposes.

11.3.3 (Recommended) photomicrographs documenting representative examples of minerals and other components of the sample.

11.3.3.1 Photomicrographs should include a scale or equivalent information.

11.3.4 Citation of any references, databases, or physical reference materials supporting the interpretations of grains characterized by PLM.

11.4 For comparisons, document similarities and differences among samples and describe the rationale for determining whether differences are explainable or exclusionary.

12. Keywords

12.1 soil; forensic analysis; polarized light microscopy (PLM), mineral grains, petrographic microscope

13. References

(1) Nesse, W. and Baird, G., *Introduction to Mineralogy,* Fourth Edition, Oxford University Press. 2023, p. 560.

(2) New York Microscopical Society, "Glossary of Microscopical Terms and Definitions," The *Microscope,* Vol 51, 1989, 2nd edition.

(3) McCrone, W. C., McCrone, L. B., and Delly, J. G., *Polarized Light Microscopy*, McCrone Research Institute, 2006, p. 251.

(4) ENFSI, *Best Practice Manual for the Forensic Comparison of Soil Traces*, ENFSI-BPM-APS-0Version 1 - December 2019, https://enfsi.eu/wp-content/uploads/2017/06/ENFSI_BPM_APST_Soil_Examination-vs1.0.pdf.

(5) Fitzpatrick, R. W., and Raven, M. D., "Guidelines for Conducting Criminal and Environmental Soil Forensic Investigations (Version 10.1)," *Report CAFSS_076*. Centre for Australian Forensic Soil Science (CAFSS), Adelaide, Australia, 2016, p. 46.

(6) Ruffell, A., Pirrie, D., and Dawson, L., "Geological Evidence Analysis," Chapter 6 of *A Guide to Forensic Geology,* Donnelly, L., Pirrie, D., Harrison, M. A., Ruffell, A., and Dawson, L. eds., Geological Society, London, 2021, pp. 129-155, DOI.org/10.1144/GFG.6\.

(7) Mange, M. A., and Maurer, H. F., *Heavy Minerals in Colour,* 1st ed., Chapman & Hall, 1992, p. 147.

(8) Heinrich, E. W., *Microscopic Identification of Minerals*, McGraw-Hill, 1965, p. 414.

(9) Bloss, F. D., *Optical Crystallography*. Mineralogical Society of America, 1999. p. 239.

(10) Deer, W. A., Howie, R. A., and Zussman, J., *An Introduction to the Rock-Forming Minerals*, 3rd Edition, Longman, 2013, p. 510.

(11) Ehlers, E. G., *Optical Mineralogy. Theory and Technique*, Blackwell Scientific, 1987, 172 p.

(12) Palenik, S., "Heavy Minerals in Forensic Science," Chapter 37 in *Heavy Minerals in Use*, Eds., Mange M., and Wright, D., Developments in Sedimentology, 2007, pp. 937-961.

(13) Soil Survey Staff, *Kellogg Soil Survey Laboratory Methods Manual*, Version 6.0, section 7B "Optical analyses", Soil Survey Investigations Report No. 42. U.S. Department of Agriculture, Natural Resources Conservation Service, 2022, pp 767-791.

(14) Bernhard, P., *The Ore Minerals Under the Microscope: an Optical Guide*, Elsevier, 2016, p 894.

(15) Vaughan, D. J. and Craig, J. R., *Ore Microscopy and Ore Petrography*, John Wiley & Sons Ltd., New York, 1994, p. 324.

(16) Wilcox, R. E., "Refractive Index Determination Using the Central Focal Masking Technique with Dispersion Colors," *American Mineralogist*, Vol 68, 1983, pp. 1226-1236.

(17) McCrone, W. C., "Detection and Identification of Asbestos by Microscopical Dispersion Staining." in *Environmental Health Perspectives* Vol 9, 1974, pp. 57-61.

(18) OSHA. *Polarized Light Microscopy of Asbestos*, ID-191, Occupational Safety and Health Administration. 1992. Available online: https://www.osha.gov/sites/default/files/methods/osha-id191.pdf

(19) NIOSH," Asbestos (bulk) by PLM, *Meth*od #9002," in *NIOSH Manual of Analytical Methods (NMAM), 5th Edition*, https://www.cdc.gov/niosh/docs/2003-154/pdfs/9002.pdf

(20) Pye, K., *Geological and soil evidence: forensic applications*, CRC press, 2007, p. 360.

(21) Szmańda, J. B., and Witkowski K., "Morphometric Parameters of Krumbein Grain Shape Charts—A Critical Approach in Light of the Automatic Grain Shape Image Analysis," *Minerals*, Vol 11, No 937, 2021,937. https://doi.org/10.3390/min11090937

(22) Cady, J. G., Wilding L. P., and Drees, L.R., "Petrographic Microscope Techniques," chapter 8 in *Methods of Soil Analysis, Part 1. Physical Mineralogical Methods*, Agronomy Monograph no. 9 (2nd Edition), American Society of Agronomy-Soil Science Society of America, 1986, pp. 185-218.

(23) Lynn, W., Thomas, J. E., and Moody, L. E., "Petrographic Microscope Techniques for Identifying Soil Minerals in Grain Mounts," in *Methods of Soil Analysis Part 5—Mineralogical Methods* SSSA, Eds., Ulery, A.L., and Drees, L. R., 2008, pp. 161-190.

(24) Graves, W. J., "A Mineralogical Soil Classification Technique for the Forensic Scientist," *Journal of Forensic Sciences*, Vol 24, 1979, pp. 323-338.

(25) Bowen, A.M., "Forensic Applications of Foraminifera," *The Microscope*, Vol 58, 2010, pp. 3-18.

(26) Isphording, W., "Forensic Use of Heavy Minerals in Civil and Criminal Investigations," Ch. 38 in Developments in Sedimentology *Heavy Minerals in Use*, Eds., Mange M., and Wright, D., 2007, pp. 963–982.

(27) Chayes, F., *Petrographic Modal Analysis An Elementary Statistical Appraisal*, John Wiley and Sons, 1956, p. 113.

(28) Dryden, A. L., "Accuracy in Percentage Representation of Heavy Mineral Frequencies," *Proc. Nat. Acad. Sci.,*Vol 17, 1931, pp. 233-238.

(29) National Cooperative Soil Survey, *National Cooperative Soil Survey Soil Characterization Database*, http://ncsslabdatamart.sc.egov.usda.gov/

(30) Stern, L. A., Webb, J. B., Ingham, J., Monteith, S., and Saginor, I., "Soil Survey Laboratory Grain Count Data to Substantiate the Rarity of Mineral Grains in Forensic Soil Reports of Examination," *Journal of Forensic Sciences*, Vol 66, 2021, pp. 2413-2423. https://doi.org/10.1111/1556-4029.14816

(31) Dunkl I., von Eynatten H., Andò S., Lünsdorf K., Morton A., Alexander B., Aradi L., Augustsson C., Bahlburg H., Barbarano M., and Benedictus A., "Comparability of heavy mineral data–The first interlaboratory round robin test," Earth-Science Reviews, 2020, Vol 211, p. 27. https://doi.org/10.1016/j.earscirev.2020.103210.

(32) Fitzpatrick, R., Raven, M., and Self, P., "The Role of Pedology and Mineralogy in Providing Evidence for 5 Crime Investigations Involving a Wide Range of Earth Materials," *Episodes: Journal of International Geoscience*, Vol 40, 2017, pp. 148-156.

(33) Bowen, A. and Caven E., "Forensic Provenance Investigations of Soil and Sediment Samples," in *Environmental and Criminal Geoforensics*, Geological Society of London Special Publications, Vol 384, 2013, pp. 9-25. 10.1144/SP384.4.

(34) Stoney, D. A., Bowen, A. M., Bryant, V. M., Caven, E. A., Cimino, M. T., and Stoney, P. L., "Particle Combination Analysis for Predictive Source Attribution: Tracing a Shipment of Contraband Ivory," *JASTEE*, Vol 2, pp. 13-73.

(35) Palenik, S.J., "The Determination of Geographical Origin of Dust Samples," in *The Particle Atlas* Vol. V, Eds. McCrone, W.C, Delly, J. G., and Palenik, S. J., Ann Arbor Science Publishers, Ann Arbor, Michigan, 1973, pp. 1347-1361.

(36) Ross, C., "The Dark-Field Stereoscopic Microscope for Mineralogic Studies," *American Mineralogist*, Vol 35, 1950, pp. 906-910.

(37) Soil Science Division Staff “Examination and Description of Soil Profiles,” Ch. 3 in Soil Survey Manual, United States Department of Agriculture Handbook No. 18, 2017, pp. 83–233. https://www.nrcs.usda.gov/resources/guides-and-instructions/soil-survey-manual

FIGURES

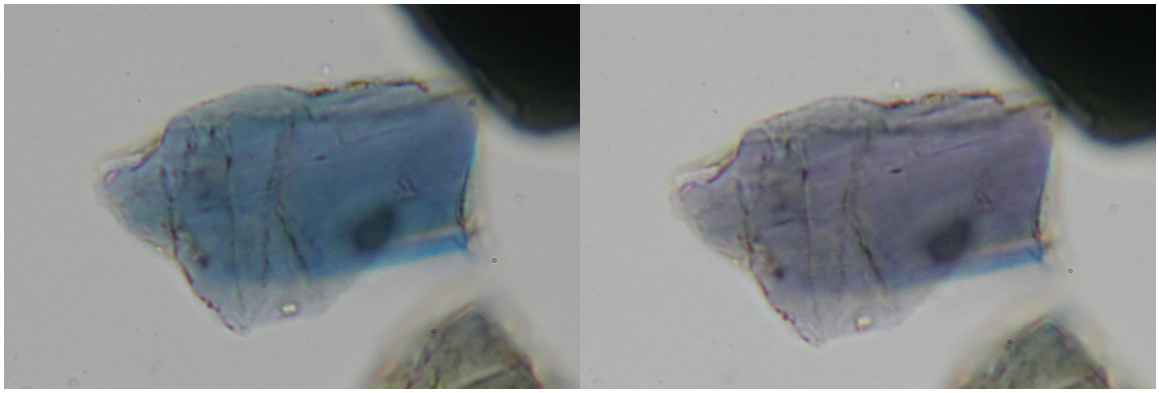

Figure 1. Grain of glaucophane mounted in 1.660 index of refraction oil and viewed in transmitted, plane polarized light. Observe the blue to purple pleochroism when the grain is mounted parallel to the polarizer (left) and perpendicular (right). Field of view is approximately 100 µm. Color of a grain can be useful for characterization and identification. Images from Ethan Groves.

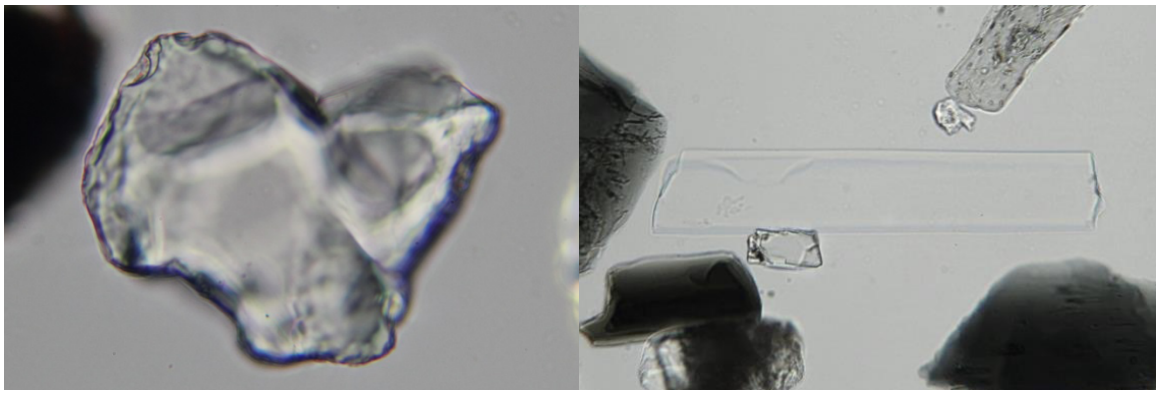

Figure 2. Examples of high and low relief: anhedral garnet exhibiting high relief (left) and a euhedral sillimanite grain showing low relief (right). Each are mounted in 1.660 index of refraction oil and viewed in transmitted, plane polarized light. Field of view is approximately 100 µm. Images from Ethan Groves.

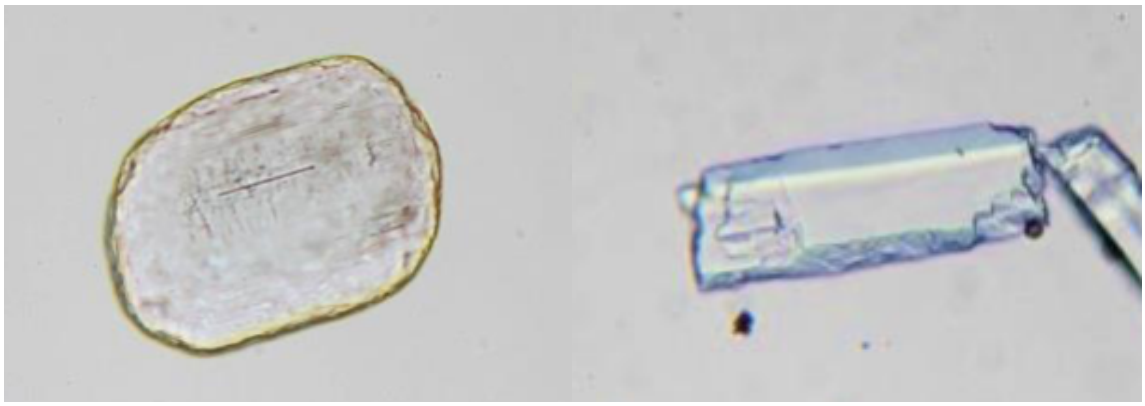

Figure 3. Yellow dispersion colors observed on the rounded apatite grain (left) and blue dispersion colors observed with the crushed kyanite grain (right). Each is mounted in 1.660 index of refraction oil and viewed in transmitted, plane polarized light. The consistent use of mounting media of a single RI allows for dispersion colors to become familiar for certain minerals and thus can be utilized as a diagnostic characteristic. Field of view is approximately 100 µm. Images from Ethan Groves.

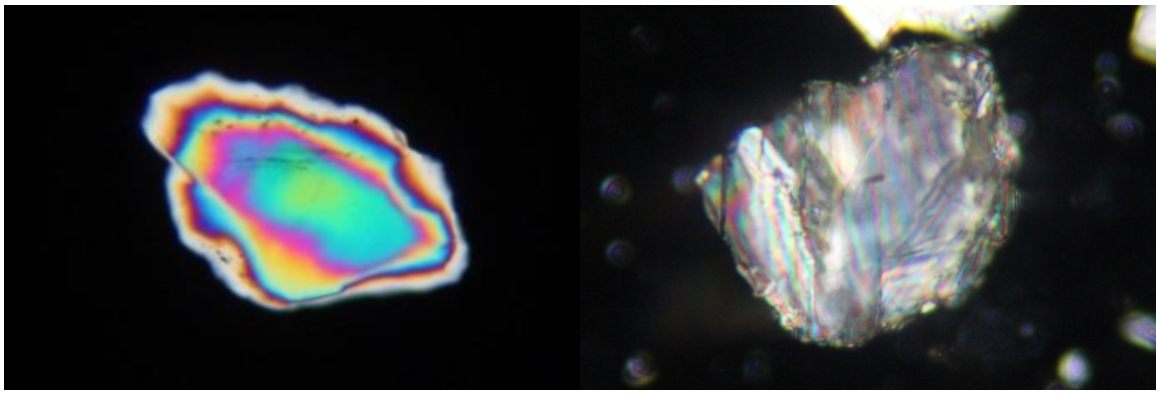

Figure 4. Examples of low order interference colors (left) and higher order colors (right). Each grain is viewed between crossed polarizers. Field of view is approximately 100 µm. Images from Ethan Groves.

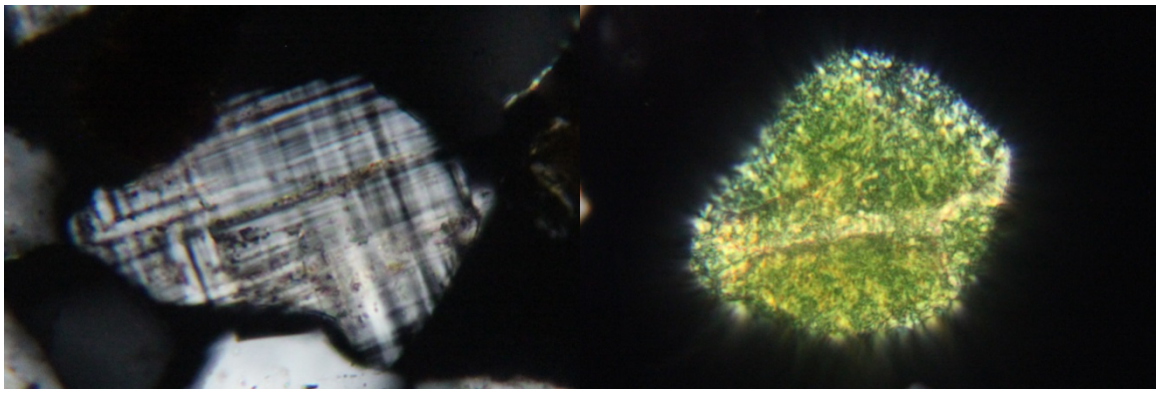

Figure 5. Right-angle twinning observed in microcline (left) and the polycrystalline character of glauconite (right) are two examples illustrating the utility of extinction. Each is mounted in 1.540 index of refraction oil and viewed between crossed polarizers. Field of view is approximately 100 µm. Images from Ethan Groves.

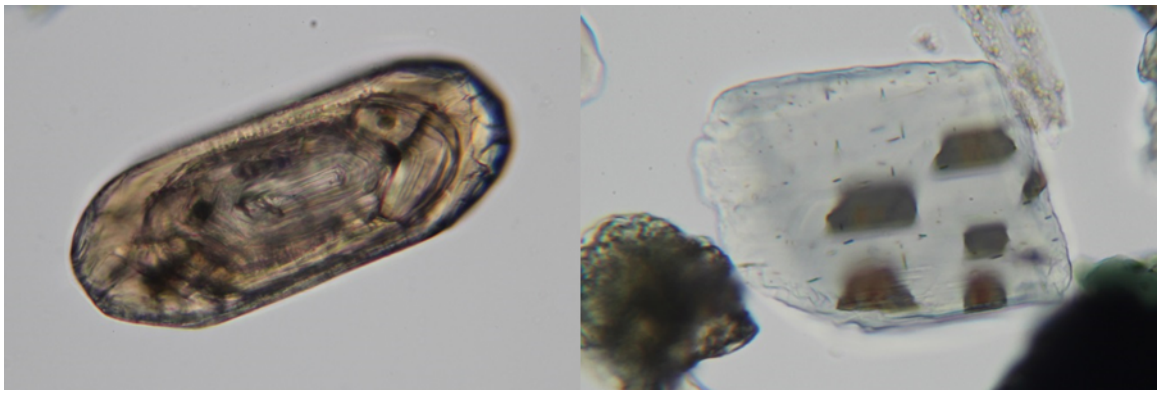

Figure 6. Zoned zircon (left) and a pyroxene with colored inclusions (right). These characteristics assist with characterizing and further distinguishing between examples of these minerals. Each is mounted in 1.660 index of refraction oil and viewed in transmitted, plane polarized light. Field of view is approximately 100 µm. Images from Ethan Groves.

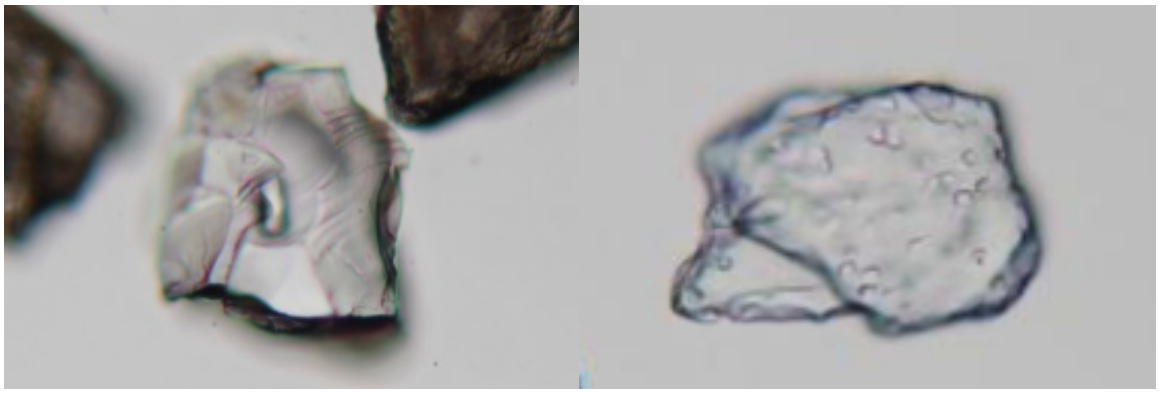

Figure 7. Fracture marks on a quartz grain (left) and dissolution pits on a garnet (right). These features are a result of the geological history or local environment from which the grains originated. Each is mounted in 1.660 index of refraction oil and viewed in transmitted, plane polarized light. Field of view is approximately 100 µm. Images from Ethan Groves.