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Training, Research, Analysis, Consulting, Education

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Training Exercise - Terminology 1

The following exercises are designed to provide a practical, working understanding of terms and simple concepts you will need to make basic observations with the polarized light microscope (PLM).

The **terms you will explore** in Terminology 01 Exercise (T1) are:

1) Isotropic
2) Becke Line
3) Relief/Contrast
4) Anisotropic
5) Refractive index (RI)
6) Mounting Medium
7) Polarizer
8) Analyzer
9) Crossed Polars
10) Focusing Up
11) Extinction
12) Birefringence

This exercise has four sections. In each section you will find instructions on how to use the prepared slides (in the slide box provided to you) to observe the particle(s) on the slide in order to view the optical property of interest. All mineral specimens are mounted in Melt Mount, which has a refractive index of 1.662 (notated as n_D = 1.662 MM).

Carefully observe the relevant optical property and document other observations for each sample listed in the section – that includes sketches **(only as needed)** and written case notes. Understanding the concept is important with not as much documentation.

Please use your own paper (8.5" x 11"; blank, lined, or graph) to record your observations. Only write on one side of the page.

Before You Begin – Align and establish Koehler illumination on the PLM

1. First look to see that the illumination is at a minimum. Turn on the microscope illuminator then adjust.

2. **Inspect** the slides in your box.
    a. If you find slides that are chipped or broken, inform an instructor or CA/TA.
    b. Check the slides you will use today for fingerprints or smudges. Use rubbing alcohol (in spray bottle) and a Kimwipe® to clean any dirty slides.

Isotropic and Anisotropic Particles

Transparent particles at the microscopic level come in two varieties: isotropic and anisotropic. The molecular makeup of an isotropic particle is such that plane polarized light (PPL) finds identical velocity through the particle regardless of the particle's orientation. Put another way, the light travels identically

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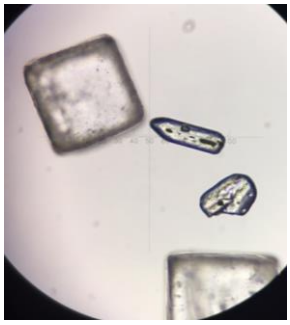
(the same speed) in every direction so the refractive index is identical in every direction; therefore, **isotropic particles have only one refractive index (RI)**.

Isotropic particles have no birefringence. **The isotropic particle remains dark when the polarizer & analyzer are crossed (cross your polars or crossed polars)**. Anisotropic particles will be "bright" in their interior when not at extinction (See figure 2). Do not mistake reflections and/or inclusions within the particle for brightness. Before deciding whether a particle is isotropic or anisotropic, rotate the stage at least 90˚. If an anisotropic particle is at an extinction position, the particle will appear dark until rotated to a bright position. The isotropic particle shows a single RI regardless of orientation – that means the particle will stay dark no matter the rotation of the stage.

Because isotropic particles have one RI, their relief does not change as you rotate the stage. This means that the contrast between the edge of the particle and the mounting medium should not change. The relief changes – although sometimes subtly – for an anisotropic particle when you rotate the stage depending on orientation.



← Figure 1. Table salt (large cubes) and zircon viewed in plane polarized light (PPL).

Based on the information you have read, which appears to be isotropic - table salt or zircon?

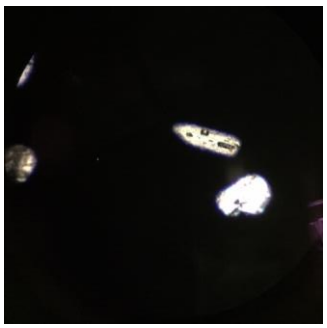


Figure 2. Table salt (dark) and zircon (bright) viewed with analyzer inserted (crossed polars).

Note that the whole interior of the zircon is bright not just inclusions inside the crystal.

Observe and document the following four slides in order:

1) Fluorite (02)	2) Obsidian (03)	3) Calcite (05)	4) Halite (01)
Calcium Fluoride	Silicon dioxide	Calcium Carbonate	Sodium chloride

Start with fluorite, then observe obsidian, then calcite, and last halite (table salt).

Procedure

1. Turn on the illuminator on the microscope.
2. Obtain a prepared slide (one of the four (4) above) from the slide box then place the prepared slide on the stage.
3. Using the 10x objective, focus on a particle on the slide in the field of view. Use of a single polar at the condenser is considered plane polarized light (PPL) viewing. (Similar to brightfield)

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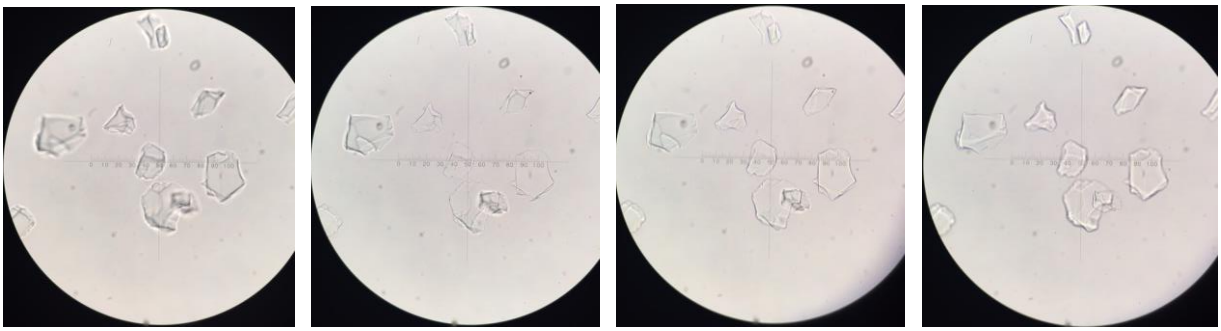
4. Put in the analyzer (cross your polars) to make the field of view dark (crossed polars). If not black, check that the analyzer is in the proper orientation.
5. Observe the interior of the specimen through the oculars. Is the center dark or bright? Slowly rotate the stage at least 90° then a full 360°.
    a. Observe the lack of colors/brightness (retardation) in the interior of the particle(s) for the isotropic particles and observe the interior brightness of the anisotropic.
    b. NOTE: Reflections of light off the outside surface or small inclusions do not count as retardation (brightness) in the particle.
6. Look at the edge of the particle to observe the relief (the darkness or apparent thickness of the edge of the particle – more on this in a later section).
7. Rotate the stage while observing the particle. Note that the relief or contrast for an isotropic particle does **not** change as you rotate the specimen, but may change for an anisotropic particle (sometimes the change may be subtle or significant).

Becke Line

The ***Becke*** line is a "halo" that can be observed around the particle. The Becke line is a result of the refraction of light through an immersed particle in a liquid. When increasing the distance between the crystal and the objective (known as "focusing up"), the Becke line ALWAYS moves into the medium of higher refraction. (that is important!)

If the Becke line (white halo) moves from the exterior to the interior of the crystal (xtl) when focusing up (F↑), which has the higher RI – the particles or the mounting medium?



Observe and document the following three slides **in order**:

1) Fluorite (02)
2) Glass (08) (n_D = 1.625)
Zircon (06)

Start with fluorite, then examine glass (nD = 1.625), and finally zircon. The Becke line informs the microscopist which media has the higher refractive index by moving into the medium with a higher index, whether the crystal or the liquid has the higher index.

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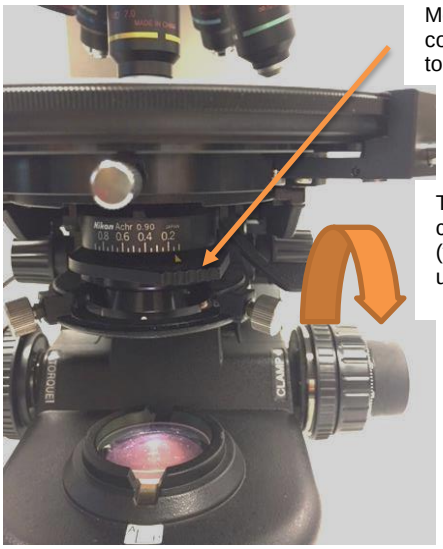
Simple right? The Becke lines moves into the index of higher refraction when focusing up!

Procedure

1. Using the 10x objective, place a prepared microscope slide on the stage. Focus on the particles on the slide in the field of view.
2. Close down the substage condenser to its smallest opening - maximizing contrast. This will make it easier to see the Becke line.
3. To observe Becke line movement, look through the oculars and "focus up" (increase the distance between the specimen and the objective on the slide) following this procedure:
    a. To focus up, put your hand on the focus knob with your thumb at the 12 o'clock position and then turn the knob towards you (counterclockwise).
    b. You may need to focus up and down **(turn the focusing knob clockwise and counter clockwise) multiple times** to see Becke line movement.



4. Note which way the Becke line moves. When you focus up, does the Becke line move into the interior of the particle or out into the mounting medium? Write this down as follows:
    a. Use the following shorthand notation to record this observation in your case notes:
        i. Example 1: If the Becke line moves into the particle or crystal (xtl) when focusing up (F↑), notate as Bk → Xtl F↑
        ii. If the Becke line moves into the mounting medium/RI liquid (liq) when focusing up (F↑), notate in your notes as Bk → liq F↑
5. Choose a particle to focus on. Set the Becke line just outside the particle using the focus knob.
    b. While looking through the oculars and not changing the focus setting on the focusing knob, rotate the microscope stage with your left hand. Look at the Becke line around the particle as you rotate the stage. Does the Becke line change or does it stay where it started?
        i. For an isotropic particle, the Becke line will remain where it started inside or outside the particle and should not change.
        ii. For an anisotropic particle, the Becke line may not change (move from outside to inside the particle or vice versa) or it may change color when rotating the stage.

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Relief / Contrast (and Becke line Colors)

The relief (or contrast) of a particle in a mounting medium depends on the difference in optical density (refractive index) between the particle and the medium assuming a lenticular particle.

Because **isotropic** particles have **one RI**, **their relief does not change as you rotate the stage**. The contrast between the edge of the particle and the mounting medium should not change with stage rotation (e.g., when rotating the particle on the stage). With an anisotropic particle, depending on the refractive index difference, the relief may change subtly or drastically when you rotate the stage depending on orientation.

When the difference in refractive index between the particle and the mounting medium is large, the relief is considered heavy. Heavy relief is characterized by a dark, thick particle edge. When the difference in refractive index between the particle and the mounting medium is small, the relief is considered light. Light relief is characterized by a thin or nearly invisible particle edge. If the edge is nearly vertical, then higher contrast will be seen – look for a thin edge or lenticular edge.

Figure 5 – Particle edge types

The decision about the relief is subjective but most students seem to come to a collective similar description of the relief. The description is something you will figure out as you examine more particles.

Here are the different relief descriptions used by many:

Very heavy	thick black line	Extremely different RI
Heavy	moderate black line	Far apart RI
Moderate	black line	Moderately different RI
Light	thin dark gray-black line	Approaching RI
Very light	hairline gray to black line	Close RI but not same
Nearly disappears	barely able to see the particle	Nearly the same RI
Virtually disappears	almost disappears in the medium	Virtually the same RI

Relief provides information about how far apart the particle and the mounting medium refractive indices are from each other. You will see high relief when the particle is much greater than the mounting

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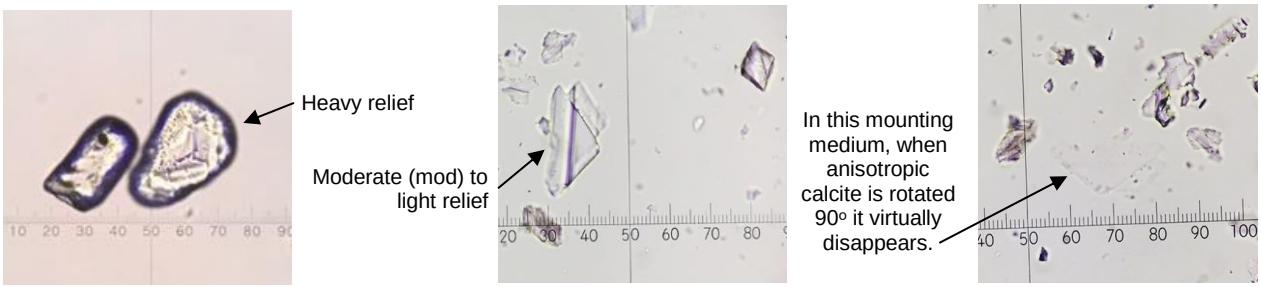
medium or when the particle is much less than the mounting medium. When the relief nearly disappears the refractive indices are nearly the same. Additional work may be necessary to bracket the refractive index or determine the refractive index.

The takeaway from this portion of the exercise is simply that the relief guides you in a qualitative way how far apart the particle and the known refractive index of the mounting medium are. As the RI of the specimen gets closer to the RI of the mounting medium, the relief gets lighter. If the RI of the specimen is the same as the RI of the mounting medium, the edge of the particle virtually disappears from view. The particles (crystals) become very difficult to find when the RIs of the specimen and the mounting medium are the same. When the RI are close, then subtle colors may be observed in the Becke line (but **not** the often reported purple and green!) The colors will be discussed more later.

Note: Keep the difference between the RI of the particles and the RI of the mounting medium in mind as you make your observations.



Observe and document the following six slides in order:

1) Fluorite n_D = 1. 433 (02)
2) Cryolite n_D = 1.34 (07)
3) Glass n_D = 1.625 (08)
4) Glass n_D = 1.66 (09)
5) Glass n_D = 1.69 (10)
6) Calcite (05)

Mounting medium refractive index is n_D = 1.660

Procedure

1. Turn on the microscope (if off) and use the 10x objective.
2. Place the appropriate prepared microscope slide on the stage [start with 1) Fluorite, then 2) Cryolite, etc.]
3. Having the 10x objective in the optic axis and while looking from the side of the stage, place the prepared slide on the stage and move the coverslip portion of the slide under the objective.

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4. **While still looking from the side,** rack the stage to its highest point but DO NOT ALLOW THE OBJECTIVE AND COVERSLIP TO TOUCH! Once at the top look through the oculars.
5. While looking through the oculars, slowly begin lowering the stage to focus on a particle on the slide in the field of view. If you do not see particles of interest, move the slide around using the one-hand technique until particles of interest come into view (not dust or artifacts on the top of the coverslip). Be sure to continuously adjust the focus as needed. This is considered plane polarized light (PPL) viewing. (Similar to brightfield)
6. **Reopen the substage condenser diaphragm to 2/3 open** as used for proper Kohler illumination. When **looking at relief, you should start with a bright field view using PPL with the condenser diaphragm at 2/3 open**.
7. Observe the relief of the particle as you rotate the stage 180°. If the particles are isotropic (or nearly so), the relief should not change.
    a. Decide whether the relief looks (see above for appearance)
        - very heavy,
        - heavy,
        - moderate,
        - light,
        - very light,
        - nearly disappears,
        - virtually disappears.
        - These are the six descriptions of relief to use to describe what is seen.

    Make sure your observation makes sense with the refractive indices between the particles and the medium – check your observation against the values of the sample versus the mounting medium.
8. Observe the Becke line of each particle observed by **focusing up**. To enhance contrast, close down the substage condenser. Which way does the Becke line move?
    a. If the RI of the particles on the slide is close to the RI of the mounting medium, you may not see any relief but you may see colored Becke lines.

Did you see any faint colors in the Becke line? (NOT Green and Purple which many students report)

a. If the RI of the particles on the slide is close to the RI of the mounting medium, you may not see any relief but you may see colored Becke lines.
b. Some **color combinations** you may see are
    - yellowish-white and dark violet,
    - lemon yellow and royal blue,
    - “burned” orange and sky blue
    - red-orange to brown and bluish-white
    - Other colors in between these color combinations may also be seen.

For Terminology 1, if you think you are observing Becke line colors, have an instructor confirm what you are seeing.

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Additionally perform the following:

9. At the current opening of the substage condenser diaphragm (2/3 open) observe the Becke line of the particles by focusing up. Document the ease or difficulty to observe the Becke line at this opening as well as the direction of the Becke line using the abbreviated note taking.
10. Close down the substage condenser diaphragm to the minimum opening.
11. Document the ease or difficulty to observe the Becke line at this opening as well as the direction of the Becke line using the abbreviated note taking.
12. Which way does the Becke line move for each particle? (Keep in mind the xtl vs liq RI)
13. When you document observations of relief or colored Becke lines in your notes, you add them to the Becke line shorthand you already learned.
    a. If a particle's Becke line goes into the liquid when focusing up and has heavy relief, write: Bk → liq F↑ heavy relief.
    b. If a particle's Becke line goes into the crystal when focusing up and has light relief, write: Bk → xtl F↑ light relief.
    c. If a particle's Becke line is colored sky blue and orange, the relief (edge) virtually disappears, and the sky blue Becke line goes into the crystal when focusing up, write: Bk → xtl F↑ virtually disappears - orange/sky blue.

Anisotropic Particles

The light refracting through transparent anisotropic particles at the microscopic level breaks into two rays of preferred vibrational directions passing through the particle. The velocity difference of the two rays results in one traveling slower than the faster. Terminology 2 explores this phenomenon in more depth. The difference between the two rays results from one ray being retarded (interacting more with atoms) in its velocity relative to the other ray (interacting less) and that difference is observed in the bright interior of the particle. The two rays may have very different velocities (*very high* ***retardation*** *color*) to nearly the same velocities (*very low retardation colors*) through the particle which is also known as the ***birefringence*** which does not change in a substance. Each substance has its own birefringence as a result of the different velocities of light. **Anisotropic particles have either two or three refractive indices**.

Anisotropic particles show retardation colors because of at least two refractive index values present. Anisotropic particles will be "bright" in their interior when not at extinction. Do not mistake reflections and/or inclusions within the particle for brightness. Before deciding whether a particle is isotropic or anisotropic, rotate the stage at least 45˚. If an anisotropic particle is at an extinction position, the particle will appear dark until rotated to a bright position.

Observe and document the following three slides in order:

1) Quartz (04) 2) Zircon (06) 3) Calcite (05)

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Procedure

1. Turn on the microscope (if off).
2. Having the 10x objective in the optic axis and while looking from the side of the stage, place the prepared slide on the stage and move the coverslip portion of the slide under the objective.
3. While stilling looking from the side, rack the stage to its highest point but DO NOT ALLOW THE OBJECTIVE AND COVERSLIP TO TOUCH! Once at the top look through the oculars.
4. While looking through the oculars, slowly begin lowering the stage to focus on a particle on the slide in the field of view. If you do not see the particles, move the slide around using the one-hand technique until particles of interest come into view. Use of a single polar at the condenser is considered plane polarized light (PPL) viewing. (Similar to brightfield) Looking for bubbles in the medium is a good way to know you are at the correct level where your particles exist.
5. Reopen the substage condenser diaphragm to 2/3 open as used for proper Kohler illumination. When looking at anisotropicity, you should start with a bright field view using PPL.
6. Look at a thin edge of the particle to observe the relief (the darkness/thickness of the edge of the particle) in plane polarized light (PPL).
7. Rotate the stage 360˚ while observing the particle. Note the change in relief (if any) as the particle rotates.
    a. The change in relief may be subtle if the difference between the two refractive indices of the particle (the birefringence) is small.
    b. If the birefringence is high, the relief of the particle should change more dramatically as the stage is rotated.
8. Put in the analyzer (cross the polars) to make the field of view dark.
9. Observe a specimen through the oculars. Bring your attention to a bright particle.
10. Rotate the stage until that particle goes dark – this is the particle's ***extinction position***.
11. Remove the analyzer (uncross the polars) and note the relief of the particle in PPL. Very important that you observe relief when the substage condenser diaphragm/aperture is 2/3 open.
12. Observe the Becke line of the particle – *focus up* (you may have to focus up and down several times on the particle to verify the *Becke line* direction) but note the direction of the Becke line while *focusing up*.
13. Cross the polars again and rotate the stage to the next extinction position (90°). Do this slowly.
    a. The particle you are observing should get bright as you approach 45° of rotation and then dim as you approach 90°. Do not read the stage numbers – use your eyes.
14. Uncross the polars and again observe and document the relief and Becke line of the particle.
    a. Did it change? Is it heavier or lighter? What does this tell you about the RIs of the particle?

POST EXERCISE QUESTIONS

Answer the questions below only after you have completed the procedures above. The questions are **specific to each section** of the exercise, so only discuss the specimens examined from that particular section and your observations made in each section in your answer to the following questions.

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Isotropic and Anisotropic Particles

1) Why are isotropic particles dark with crossed polars?
2) Which of the slides (use subject names) that you observed had particles with brightness in their interior? (Not inclusions)

Becke Line

3) When focusing up, indicate the direction of the Becke line for each of the samples you examined. Use the shorthand notation. List each slide then the abbreviation of movement.
4) For each of the particles, note which index is higher as a result of the Becke line movement.
5) Which particles had white Becke lines? Which had colored Becke lines? Why did some have color lines while others were white? (no slide has green and purple Becke lines!)

Relief

6) Which particle had the highest relief (darkest edge)? What was the difference in refractive index between the mounting medium and the index listed for the particle?
7) Which particle had the lowest relief? For the lowest relief particle, what was the difference in refractive index between the mounting medium and the index listed for the particle?
8) Which particles are easier to find, those with heavy relief or those with light relief?
9) Describe the relief of the calcite particle at the two extinction positions. (Be sure you use a rhomb shaped particle for the exercise. How many degrees did you rotate the stage to move from one extinction position to the next?