General Microscopy Techniques

1 Scope

Personnel in the following disciplines: Hairs and Fibers, Geology, and Anthropology may use general microscopy techniques during their examination of evidence. Due to the wide variety of requests and examinations, this procedure provides general guidelines for general microscopy techniques and may or may not apply to every microscope used in each discipline. This document applies to personnel within the Trace Evidence Unit (TEU) and Scientific and Biometrics Analysis Unit – Trace.

2 Equipment/Materials/Reagents

- Centering wrenches to fit microscope of interest
- Chemical resistant gloves
- Dust covers for microscopes
- Inert dusting gas
- Laboratory coat
- Lens cleaner
- Lens paper
- Petrographic microscope, with minimum 4x objective, 7.5x eyepieces rotatable stage, lower condensing lens
- Phase contrast microscope with minimum 10x magnification
- Prepared slide of particulate material immersed in mounting media with cover slip
- Sable brush
- Stereobinocular microscope, with minimum magnification of 4 diameters
- Water
- White polystyrene foam (small grain type)

3 Standards and Controls

Not applicable.

4 Sampling

Not applicable.

5 Procedure

5.1 General Care and Maintenance of Microscopes

The optical components of the microscopes should be kept clean at all times.

5.1.1 Replace the dust cover on the microscope at the end of each day, when available.

5.1.2 Clean the external surfaces of objectives, eyepieces and condensers.

5.1.2.1 Gently blow dust away, using a can of inert dusting gas, or using a clean soft sable brush.

5.1.2.2 After blowing away dust, use lens paper, dampened with water or lens cleaner to remove fingerprints, grease, oil and dirt. Never use alcohol on lenses.

5.1.3 White polystyrene foam (small-grain type) is recommended by some microscope companies for removing residues of immersion oil, skin grease and solvents. Break off a small piece and press a projecting part of it against the dry lens, rotating it as co-axially as possible with the objective. Any adhering foam granules can then be removed by blowing them away or using an absolutely clean sable brush.

5.1.4 Special care is required when working with acids and other chemical reagents. Their contact with the objectives should be strictly avoided. Clean the objectives (and other contaminated areas) at once after any accident. Even when particles are under a cover glass, there is a continuous stream of vapor from the corrosive substances that will impair the optical quality of the objective lens. Do not subject the lenses to prolonged exposure to these vapors.

5.2 Microscope Alignment

Modified Köhler illumination is used in petrographic and phase contrast microscopes.

5.2.1 Fully open field and aperture diaphragms, if present.

5.2.2 Adjust interpupillary distance (IPD).

5.2.3 Place specimen on stage.

5.2.4 Using only one eyepiece focus on the preparation using fine and coarse adjust controls on microscope. Adjust other eyepiece so that it is parfocal with the first.

5.2.5 For a Microscope Containing an Eyepiece Graticule

5.2.5.1 Without looking into the eyepieces, turn the eyelenses fully counterclockwise until they stop.

5.2.5.2 While looking into the eyepiece containing the graticule, adjust the eyepiece containing the graticule until the graticule comes into focus.

5.2.5.3 Do not adjust the eyepiece containing the graticule again.

5.2.5.4 Using the eyepiece containing the graticule, focus on the test object.

5.2.5.4.1 Place a flat object beneath the objective.

5.2.5.4.2 Using the lowest magnification and the eyepiece containing the graticule, bring the object into focus.

5.2.5.4.3 Using the highest magnification, bring the image into precise focus.

5.2.5.5 Adjust the eyepiece which does not contain the graticule.

5.2.5.5.1 Using the lowest magnification and the eyepiece which does not contain the graticule, focus the eyelens.

5.2.5.5.2 Using the highest magnification, look at the object with both eyepieces, and bring the image into precise focus.

5.2.6 For a Microscope that Does Not Contain an Eyepiece Graticle

5.2.6.1 Adjust the right eyepiece until it is in the center of its focusing range. Do not adjust the right eyepiece again.

5.2.6.1.1 Focus on the test object.

5.2.6.1.1.1 Place a flat object beneath the objective.

5.2.6.1.1.2 Using the lowest magnification and the right eyepiece bring the object into focus.

5.2.6.1.1.3 Using the highest magnification, bring the image into precise focus.

5.2.6.2 Adjust the left eyepiece

5.2.6.2.1 Without looking into the eyepiece, turn the eyelens fully counterclockwise.

5.2.6.2.2 Using the lowest magnification and the left eyepiece, focus the eyelens.

5.2.6.2.3 Using the highest magnification, look at the object with both eyepieces, and bring the image into precise focus, if necessary.

5.2.7 For a Microscope that Includes a Substage Assembly

5.2.7.1 Close field diaphragm by approximately one-half (1/2).

5.2.7.2 Focus and center condenser.

5.2.7.2.1 To focus the condenser, adjust the condenser focus knob until the image of the field diaphragm is in sharp focus.

5.2.7.2.2 To center the condenser, adjust the condenser centering screws until the image of the field diaphragm is centered in the field of view.

5.2.7.3 Open field diaphragm until just out of view.

5.2.7.4 Remove eyepiece and open aperture diaphragm until just out of view. Replace eyepiece.

5.2.8 For a Microscope with Adjustable Objectives

5.2.8.1 Place a prepared slide containing small particles on the microscope stage.

5.2.8.2 Using the lowest power objective, focus on a single particle.

5.2.8.3 Move the slide so that the particle of interest is in the center of field of view.

5.2.8.4 Rotate the stage. The particle should remain in the center of rotation. If the particle moves away from the center of the rotation:

5.2.8.4.1 Rotate the stage until the particle is furthest from the center of the field of view.

5.2.8.4.2 Adjust the objective centering wrenches until the particle is half way between its original position and the center of the field of view.

5.2.8.4.3 Repeat steps 5.2.8.4 through 5.2.8.4.2 until the particle remains in the center of the field of view upon rotation of the stage.

5.2.8.5 Repeat steps 5.2.8.2 through 5.2.8.4.2 for the remaining objectives.

5.2.9 For Microscopes with Phase Rings

5.2.9.1 If possible, align light source so that light from sub-stage is optimized while viewing through the microscope without a specimen on the stage.

5.2.9.2 Fully open the iris diaphragm.

5.2.9.3 With the two captive centering wrenches withdrawn, rotate the knurled turret ring to the open setting. These wrenches must be withdrawn to permit turret rotation.

5.2.9.4 Place a slide with mounted small particles on the stage.

5.2.9.5 Loosen condenser stop retaining screw and raise condenser with focusing knob.

5.2.9.6 Using the 10x objective, focus on the particles.

5.2.9.7 Center annular rings.

5.2.9.7.1 Place specimen slide on stage and open field diaphragm until iris just disappears from field of view.

5.2.9.7.2 Bring the specimen into good focus.

5.2.9.7.3 Turn the knurled ring to the 10x annulus and push in the two centering wrenches.

5.2.9.7.4 Image the aperture and bring the annulus of the condenser and the diffraction plate of the objective into sharp focus by turning the focusing knob of the aperture viewing unit.

5.2.9.7.5 Adjust the annular diaphragm centering wrenches until the annular image is superimposed on the diffraction plate.

5.2.9.7.6 Bring the specimen into sharp focus.

5.3 Color Balancing – Comparison Microscopes

5.3.1 After performing modified Köhler illumination on both sides of the comparison microscope, place slides with colored fibers from the same known sample on both sides of the comparison microscope.

5.3.2. Adjust the light voltage (if applicable), aperture diaphragm, and field diaphragm until the background color appears the same on both sides of the comparison microscope.

5.3.3 If so equipped, adjust the Vari-Lum or the colored filters until the colors appear the same in both sides of the comparison microscope.

5.4 Microscope Transport

If items will be examined outside the Laboratory there may be a need to bring a stereobinocular microscope to the external location to facilitate the examinations.

5.4.1 The microscope will be disassembled into its component parts and carefully packed in a container (*e.g.*, Pelican case) to ensure they will not be damaged during transport.

5.4.2 The component parts will be inspected for possible damage and the microscope will be reassembled at the external location. If any damage has occurred, the microscope will not be used.

6 Calculations

Not applicable.

7 Measurement Uncertainty

Not applicable.

8 Limitations

Not applicable.

9 Safety

No potential hazards are associated with these techniques.

10 References

- Nikon, How To Use A Microscope And Take A Photomicrograph, Nikon Corporation, 1998.
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- Delly, J.G., The Michel-Lévy Interference Color Chart-Microscopy's Color Key, *Microscope* 37, 89-102, 1989.

Rev. #	Issue Date	History
3	02/10/2020	Updated 'geological materials' to 'geologically-derived' in Scope. Updated Scientific and Biometrics Analysis Unit – Trace name. Removed 'Sample Selection' from Section 4 title. Updated language in Sections 5.3.1 and 5.3.2 for clarity. Added Section 5.4.
4	05/03/2021	Changed category of testing to discipline and updated discipline names in Scope.

Approval

Redacted - Signatures on File

Trace Evidence Unit Chief: Date: 04/30/2021

Scientific and Biometrics Analysis Unit Chief: Date: 04/30/2021

Hairs and Fibers Technical Leader: Date: 04/30/2021

Geology Technical Leader: Date: 04/30/2021

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