TES09 - Polarized Light Microscopy for Fiber Examination

Scope

This document addresses the methods for setting up the microscope and completing individual fiber examinations using the polarized light microscope. This procedure applies to fiber samples that have been previously removed from evidentiary items and have been mounted on glass microscope slides using the Evidence Handling Procedures.

Safety Precautions

No specific hazards are associated with the microscopic examination techniques performed.

Materials Required

- Polarized light microscope, magnification range from 40x to 400x, with eyepiece reticle
- Stage micrometer
- Full wave (lambda) plate
- Quartz wedge or equivalent compensator

Procedure

Microscope Preparation

Centering Stage and Objectives

The centration of the stage and objectives should be checked each time the microscope is used and should be adjusted at the discretion of the examiner.

1. With the 40x objective in place, focus on sample.
2. With the sample centered under the cross-lines of the eyepiece reticle, rotate the stage.
3. If all of the particles do not rotate about the center of the crosshairs, bring the center of rotation to the center of the crosshairs using the objective centering screws (screw-holes are located on either side of the objective on the nosepiece).
    a. Rotate the sample until it is at its maximum distance from the center of the cross-lines.
    b. With the objective centering screws bring the samples halfway from its maximum distance to the center of the crosshairs. (Do not use the stage centering screws).
    c. Repeat steps a and b until the sample rotates about the center of the cross-lines.
    d. Repeat steps 2 and 3 with the remaining objectives.
    e. Note that if you are using a microscope with a rotating stage that is not a polarizing light microscope, your objectives will not be centerable. You will need to center the stage using the stage centering screws using a procedure similar to that described above.

Setting Kohler Illumination

Kohler illumination maximizes the contrast resolution and depth of field of a sample. It should be performed every day the microscope is used.

1. Turn on the lamp.
2. Place a fiber slide on the stage.
3. Adjust the interocular distance for your eyes.
4. Place the collector lens (if present) in position.
5. With the fine focus adjustment, focus the microscope on the fiber with one ocular only. One should use one of the higher power objectives (20 x, 25x or 40x) and make sure the top lens of the substage condenser is inserted in the optical axis of the microscope.
6. Next look through the other ocular at the same particle and focus by rotating the ocular focus on the ocular tube. (Do not use the microscope fine focus adjustment).
7. Adjust the light source until the lamp filament is centered or the field of view is evenly illuminated. **Note:** Many microscopes contain a diffusing lens which prevents one from viewing the lamp filament. If this is the case the only adjustment needed is to make sure the field of view is evenly illuminated.
8. Open the aperture diaphragm.
9. Partly close down the field diaphragm.
10. Focus the edges of the field diaphragm by raising or lowering the substage condenser.
11. Center the field diaphragm by centering the substage condenser using the condenser centering screws.
12. Open the field diaphragm until the edges of the diaphragm are just outside of the field of view.
13. The proper position for the aperture diaphragm is to open it so as to occupy approximately 1/3 of the field of view for the lower power objectives. Note that the opening of the aperature diaphragm can be viewed with a Bertrand lens or by removing an eyepiece from the microscope. As you use higher power objectives you will need to open the aperature diaphragm to increase the amount of light illuminating the sample.
14. If a Bertrand Lens is present follow these additional steps:
    a. With the highest objective in place, introduce the Bertrand lens and open the aperture diaphragm.
    b. Using the fine focus adjustment, focus on the edges of the filaments.
    c. Check the centration of the filaments and adjust if needed. The filaments should completely fill the objective back focal plane.
    d. Remove the Bertrand lens and partially close the aperture diaphragm.
    e. Note that if there is a diffusing lens in the microscope that cannot be removed you will not be able to focus on the lamp filament.

Calibrating the Eyepiece Reticle

This can only be accomplished if one of the eyepieces contains an eyepiece reticle. After the initial calibration the eyepiece reticle should only need to be calibrated if a new objective or eyepiece is introduced into the system.

1. Calculate the distance in micrometers (µm)between each division on your stage micrometer. This is done by taking the length of the stage micrometer scale and dividing it by the number of divisions across its length and adjusting the units. For example: A micrometer 1mm in length containing 100 divisions:
    a. 1 mm/100 divisions = 0.01 mm per division

   b. 1 division = 0.01 mm = 10 µm
2. With the lowest objective in place, set the stage micrometer on the microscope stage and focus on the division markings.
3. Look at the far left field of view and align the stage micrometer (SM) scale with the ocular scale of the eyepiece reticle (ER).
4. Find a position on the left side of the field of view where the SM and ER divisions are aligned and record these division numbers.
5. Next look at the right hand slide of the field of view and find a position where the SM and ER divisions are aligned and record these division numbers.
6. Calculate the total number of divisions between the left and right hand aligned positions for both the SM and the ER. This is achieved by subtracting the left hand division number from the right hand division number.
7. Calculate the distance between each division:

$$1 \text{ division on the ER} = \frac{\text{Number of divisions on the SM x distance (in µm) per SM division}}{\text{Number of division on the ER}}$$

8. Repeat steps 3 through 7 for each objective.

Fiber Examinations

Individual fibers will be examined for the following characteristics:

1. Fiber Color: The examiner will document the color of the fiber and any apparent variation in the color, if present.
2. Fiber Luster: The examiner will document the presence, absence and relative abundance of delustrant particles present in manufactured fibers. This includes the size of the particles, the uniformity of the particles and the particle distribution. This characteristic applies only to manufactured fibers.
3. Optical Cross-Section: The examiner will document the apparent cross-sectional shape via optical cross-sectioning. Physical cross-sectioning methods may be used if found necessary by the examiner. Refer to TES08 for physical cross-sectioning methods. Cross-sectioning typically applies only to manufactured fibers but may be useful in the identification of certain types of natural fibers.
4. Other characteristics: The examiner will document any surface damage, manufacturing striations or other characteristics apparent in the sample.
5. Fiber Diameter: The examiner will measure the diameter (in µm) or the range in diameter of a fiber with the aid of an eyepiece reticle.
   a. Note number of eyepiece reticle divisions across the width of a fiber.
   b. Multiply the number of eyepiece reticle divisions by the distance between each division as was determined when calibrating the eyepiece reticle.
   c. If fiber diameter is variable within an individual fiber or within a sample, document the range of the diameter.
6. Becke Line and Relative Refractive Index Determination: For manufactured fibers, the determination of refractive indices relative to Permount is determined using plane polarized light. These characteristics typically only apply to manufactured fibers.

a. Close down the aperture diaphragm.
b. Orient the length of the fiber parallel to the polarizer and focus on the sample. There should be a halo of light (Becke Line) around the edges of the sample.
c. Lower the stage of the microscope (this increases the working distance and raises the focus) to observe into which substance (fiber or permount) the Becke Line appears to travel. The Becke line will move into the substance with the higher refractive index.
d. Next open the aperture diaphragm and refocus the sample. This will allow the user to observe the contrast of the sample. The greater the contrast the greater the difference in refractive indices between the mounting medium and the sample.
e. By examining the Becke Line movement and the contrast of the fiber, the relative refractive index parallel to the polarizer ($n_{\parallel}$) is determined.
f. Now rotate the stage so that the orientation of the fibers length is perpendicular to the polarizer. Repeat steps c and d to determine the relative refractive index perpendicular to the polarizer ($n_{\perp}$).
g. The isotropic refractive index can be determined by placing the fiber in any orientation under normal light, or in the 45 degree angle, in plane polarized light. Steps c and d are again repeated to determine the isotropic refractive index. This is especially important when comparing two fibers using a comparison microscope.

7. Pleochoism (Dichroism): The examiner will document the presence and/or degree of pleochroism in a sample.
    a. Under plane polarized light, rotate the fiber 360 degrees through all of its orientations. If the color of the fiber changes as its orientation changes the fiber is pleochroic.
    b. If the fiber is pleochroic, document the different colors seen at various orientations.
8. Sign of Elongation: For manufactured fibers, the sign of elongation can be determined by placing the fiber under crossed polars and introducing a compensator.
    a. Orient the fiber in the 45 degree position with respect to the polarizer.
    b. Under crossed polars observe the interference colors.
    c. Introduce a compensator
        i. For fibers that appear white, insert the full wave plate in the additive position (slow wave of the compensator is parallel to the length of the fiber).
            1. If the fiber's color does not change it has a high order white interference color and is positive.
            2. If the fiber appears blue it has a positive sign of elongation.
            3. If the fiber appears yellow or orange it has a negative sign of elongation.
        ii. For fibers that appear dark grey or black insert the full wave plate in the additive position (slow wave of the compensator is parallel to the length of the fiber).
            1. If the fiber appears blue it has a positive sign of elongation.

2. If the fiber appears yellow or orange it has a negative sign of elongation.

iii. For fibers that have interference colors other than those listed above, insert the full wave plate in the additive position (slow wave of the compensator is parallel to the length of the fiber).
   1. If the fiber color appears to move up (to the right) the Michel Levy chart, the fiber has a positive sign of elongation.
   2. If the fiber color appears to move down (to the left) the Michel Levy chart, the fiber has a negative sign of elongation.

9. Retardation (Path Difference): For manufactured fibers, the retardation (distance the slow wave falls behind the fast wave) will be estimated through the use of a Michel Levy chart and a quartz edge (or equivalent). This characteristic typically only applies to manufactured fibers.
   a. Orient the fiber in the 45 degree position to the polarizer.
   b. Under crossed polars observe the interference colors.
   c. Utilizing the Michel Levy color chart, correlate the interference color to its numerical retardation or path difference (in nanometers – nm) noted on the chart.
   d. For higher order interference colors, especially high order whites, the application of compensators may be necessary to determine an accurate path distance.
      i. Insert the quartz edge at the 45 degree angle with respect to the polarizer and analyzer.
      ii. By rotating the polarized microscope circular stage, a specimen is oriented in a subtractive alignment with respect to the compensator (slow wave of the compensator is perpendicular to the length of the positive fiber).
      iii. As the wedge compensator is slowly translated farther into the light path, the optical path difference decreases to generate a succession of interference colors. The quartz edge is inserted until the fiber appears compensated (extinct or black).
      iv. The specimen optical path difference can be estimated by comparing the black fringe appearing in the compensated fiber to the colored background fringe in next to the fiber. This colored background fringe is located on a Michel Levy chart to ascertain the optical path difference of the fiber.
10. Birefringence (B): The birefringence is the numerical difference between $n_{\parallel}$ and $n_{\perp}$.

$$B = n_{\parallel} - n_{\perp}$$

For manufactured fibers, the birefringence can be estimated by dividing the retardation, or path difference, by the diameter (thickness) of the fiber.

$$B = \text{Retardation (nm)} / \text{Thickness } (\mu m)$$

Comments

Not applicable

References

- Gaudette, B.D., The Forensic Aspects of Textile Fiber Examination. In, Forensic Science Handbook, Vol. II. R. Saferstein (ed), Prentice Hall, pp 209-272, 1988.

- Grieve, M., Fibres and Their Examination in Forensic Science, in, Forensic Science Progress, Vol. 4. A. Maehly, R.L. Williams (eds), Springer-Verlag, pp 41-126, 1990.

- Houck, Max, Forensic Fiber Examination and Analysis, Forensic Science Review, 17, No. 1, pp 30-49, 2005.

- Patzelt, W.J., Polarized Light Microscopy, Principles, Instruments, Applications, Ernst Leitz Wetzler GmbH, 1985.

- Rochow, Theodore George and Tucker, Paul Arthur, Introduction to Microscopy by Means of Light, Electrons, X rays or Acoustics, 2nd edition, Plenum Press, New York, Chapter 6, 1994.

- Warner, Steven B., Fiber Science, Prentice-Hall, Inc. 1995.

- Forensic Examination of Fibres, J. Robertson (ed), Taylor & Francis, 1999.