I. Scope:

This method describes the procedure for basic microscopy setup on compound polarized light microscopes (PLM) but certain sections can also be used for brightfield (biological) microscopes. Also, the "Eyepiece Adjustment" and the "Eyepiece Scale Calibration" sections could be used for stereomicroscopes and the "Eyepiece Adjustment" can be used for the IR microscope.

The proper setup of the microscope is important since the PLM is one of the most powerful analytical tools available to the forensic trace examiner. PLM allows an examiner to quickly characterize, identify and compare particles/fibers encountered in case work by determining a variety of physical and optical properties which includes: size, morphology, surface texture, color, pleochroism, refractive index (indices), birefringence, sign of elongation, extinction, interference figure, optic sign, and crystal system. Microchemical tests can also be conducted.

II. References:

1) Manufacturer's Instrument Manual
2) "Polarized Light Microscopy" McCrone, McCrone, and Delly (2001 or previous editions) McCrone Research Institute, Chicago, IL 60616
3) McCrone W. C., Particle Characterization by PLM: Part II Single Polar, The Microscope, Vol. 30, No. 4, 1982, p315-331
4) McCrone W. C., Particle Characterization by PLM: Part I No Polars, The Microscope, Vol. 30, No. 3, 1982, p185-196
5) McCrone W. C., Particle Characterization by PLM: Part III Crossed Polars, The Microscope, Vol. 31, No. 2, 1983, p187-206
6) SWGMAT Paint Analysis and Comparison Guidelines http://www.swgmat.org/paint.htm SWGMAT Guideline for the Forensic Examination of Pressure-sensitive Tapes - http://www.swgmat.org/tape.htm
7) SWGMAT Fiber Guidelines - http://www.swgmat.org/fiber.htm

Validation

Microscopy is a well known and scientifically accepted method for the identification, analysis, and comparison of many types of trace evidence. Relevant examples of the broad nature of the method and related literature can be found in Section II (References).

III. Safety Precautions:

Normal laboratory safety procedures should be practiced.

IV. Apparatus/Reagents:

Eyepiece Scale Adjustment

When the eyepiece scale is used for detailed and accurate measurements, it should be checked and adjusted once per year using a calibrated micrometer (stage) scale that has a known dimension. Usually for a microscope, the micrometer scale is 1 mm long divided into 100 divisions, so each division of the scale is 10 µm. The micrometer scale is placed on the stage and brought into good focus. Move the micrometer scale so it is parallel to the eyepiece scale but slightly off-set. The eyepiece scale also must be in good focus (see "Eyepiece Adjustment" below). Use as much of the length of the two scales to obtain the greatest accuracy. For example, for the 40X objective the division lines for both scales overlap in such a manner that 80 eyepiece scale divisions overlap 20 stage scale divisions. Since each stage scale division represents 10 µm, the length is 200 µm. 80 eyepiece scale divisions divided into 200 µm equals 2.5 µm per eyepiece scale division. This process must be conducted for each objective. Also, this same method is applied to the adjustment of the eyepiece scale on the stereomicroscope.

When the eyepiece scale is adjusted, it should be recorded in a logbook.

The calibrated micrometer should be taken out of service and sent out for a quality check if damage occurs.

V. Procedures:

Sampling and Sample Preparation:

Most samples should be mounted on a glass microscope slide in a suitable mounting medium. Refer to individual trace evidence sub-discipline procedures for further requirements (if any) regarding sample preparation.

Köhler Illumination and Microscope Alignment

Field Diaphragm Adjustment:
Place a prepared slide on the microscope stage and turn on the microscope. Adjust the light intensity to a comfortable level and focus on a particle with the 10X objective or an objective of higher magnification. Make sure the top condenser lens is in position and open the aperture diaphragm on the condenser. Close down the field diaphragm. Adjust the condenser height until the image of the field diaphragm (the edges of the diaphragm) is in sharp focus. Reduce color fringes to a minimum with the aperture diaphragm. Center the image of the field diaphragm by using the centering screws on the condenser. Gradually widen the field diaphragm until it leaves the field of view. Adjust the aperture diaphragm as desired for the best resolution/contrast. Re-adjust the light intensity to a comfortable level.

Lamp Adjustment:
Refer to the manufacturer's manual if the lamp on the microscope can be focused and centered.

Objective Centering:
Place a prepared slide on the stage and focus on a particle with the objective used to center the field diaphragm in the above procedure. Locate a small particle and move it under the intersection of the crosslines. Close the field diaphragm down and check to insure that it is in good focus and centered. Rotate the nosepiece to an objective having a different magnification. Center the field diaphragm using the objective centering wrenches. The particle will move as the field diaphragm moves and place the particle under the crosslines. Do this for all the objectives.

Stage Centering Procedure:
Place a slide on the stage and focus on a particle with the 10X or higher magnification objective. Locate a small particle and move it under the intersection of the crosslines. Note this as point "A". Rotate the specimen 180 degrees and this will be point "C". The center position is halfway between points "A" and point "C". Use the stage centering wrenches, move point "C" halfway to the intersection of the crosslines. Now, move a particle back under the intersection of the crosslines by moving the slide. Rotate the stage and the particle should stay under the crosslines. If the particle does not stay under the intersection of the crosslines, repeat steps until the stage is centered.

Eyepiece Adjustment:

Make sure the eyepiece with the crosslines and/or micrometer is in the right eyepiece tube and make sure the positioning pin on the eyepiece is inserted correctly in the eyepiece tube. Block the light from the left eyepiece using a piece of paper or similar object. Adjust the knurled ring on the right eyepiece and bring the crosslines and/or micrometer scale into good focus. Focus on the specimen with the 40X objective. The specimen and the crosslines and/or micrometer should both be in sharp focus through the right eyepiece. Block the light from the right eyepiece and view the specimen with the left eyepiece only. Adjust the diopter adjustment ring on the left eyepiece to bring the particle into sharp focus. Now the particle should be in good focus for both eyes. This same procedure can be used for the stereomicroscope.

Alignment of the Polarizer and the Analyzer with the Eyepiece Crosslines

Set the rotating analyzer to the "zero" mark. Place a straight synthetic fiber on the stage and bring the object into good focus. Move the slide so the fiber is directly under the intersection on the crosslines. Insert the analyzer (cross the polars) and rotate the polarizer in the condenser so the field of view is as black as possible. Rotate the fiber to extinction. Uncross the polars and check to see if the fiber is parallel with the "East-West" crossline or the "North-South" crossline. If not, carefully loosen the head of the microscope and rotate the head until the fiber is parallel to one of the crosslines. Re-tighten the head. Cross the polars and check to insure that the fiber is at extinction when parallel to the crossline. If not, repeat the steps.

V. Quality Assurance/Quality Control:

By checking for proper set-up before any examinations are conducted and through regular maintenance, and adjustments, the quality of this method is maintained.

There is no known error rate for this type of examination.