

DRUG CHEMISTRY SECTION TECHNICAL PROCEDURE MANUAL		
Procedure B-01	Polarized Light Microscopy	
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Name of Procedure:

Polarized Light Microscopy

Suggested Uses:

The polarized light microscope serves as a tool for the testing of excipients, the use of a microcrystalline test for the testing of drugs and for the determination of optical isomers.

Apparatus Needed To Perform Procedure Including Preparation of Reagent:

Polarized light microscope
Microscope slides
Objective centering screws
Spatula
Specimen

Kohler Illumination for Microscopes With a Built-in Illuminator:

1. Focus on any slide preparation of small dry particles using the coarse and fine adjustment knobs.
2. Adjust the observation tube.
 1. For one ocular tube:
 - a. Focus specimen using the fine adjustment knob.
 - b. Focus ocular cross hairs by rotating the ocular top lens.
 2. For binocular tubes:
 - a. Adjust the interpupillary distance by sliding the knurled dovetail slides of the right and left eyepiece tubes, until perfect binocular vision is obtained.
 - b. Looking through the right eyepiece (with cross hairs in view) with your right eye, rotate the upper helicoid ring of the eyepiece until the cross hairs are sharply focused.
 - c. Focus on the specimen with the coarse and fine adjustment knobs so that the sharp images of the specimen and cross hairs can be obtained simultaneously.
 - d. Now look at the image through the left eyepiece with your left eye and rotate the diopter adjustment ring to focus on the specimen without using the coarse and fine adjustment knobs.

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Kohler Illumination for Microscopes With a Built-in Illuminator (continued):

3. Center the stage rotation (with the objective centering screws) for the highest dry objective so the specimen rotates evenly about the x and y axis of the cross hairs.
4. Close the field diaphragm.
5. Make sure the image of the field diaphragm is in the center of view by adjusting the condenser using the attached condenser centering screws.
6. Focus the image of the field diaphragm by turning the substage condenser knob up or down.
7. Repeat Step 5 if necessary.
8. Open the field diaphragm to the edge of the field of view.
9. If the microscope has an adjustable lamp do the following Steps 1-4, if not, then go to Step 10.
 1. Introduce the Bertrand lens to check the focus and centration of the filament, and remove the ocular and observe the objective back focal plane.
 2. Focus image of the filament in the objective back focal plane (adjustment on lamp).
 3. Center image of the filament (adjustment on lamp).
 4. Turn Bertrand lens out.
10. Swing in the next highest objective and center it with the objective centering screws on that objective mount. Repeat this step for any remaining objectives.
11. Adjust the substage aperture diaphragm for optimum contrast and adjust the field diaphragm image size to the edge of the field of view for each objective.

Operation of the Polarized Light Microscope:

1. Switch on the light source.
2. Place the specimen slide on the stage.
3. Adjust the desired light intensity with the control lever.
4. Make sure field diaphragm is open to the edge of the field view.
5. Focus with coarse and fine adjustments for the desired objective.
6. Move the microscope slide around to view the entire specimen, adjusting the focus accordingly.
7. If the objective is changed:
 1. Adjust the fine focus adjustment.
 2. Set the field diaphragm to just inside the field of view.
 3. Adjust the aperture diaphragm for optimum contrast and resolution.

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Literature References:

“Particle Characterization by PLM, Part I No. Polarities,” Microscope, 30:3 185-196 (1982).

Instruction Manual for the Olympus Microscope, Model BHSP