



ATF-LS-E20 Basic Microscopy Setup on Compound Microscopes	Published Online: March 2018
Authority: Technical Leader	
Unofficial Copy; May Not Be Most Current Version	Page: 1 of 2

I. **Scope:** This method describes the procedure for basic microscopy setup on compound microscopes.

II. **References:**

Manufacturer's workbook

"Polarized Light Microscopy" Walter C. McCrone, et. al. Ann Arbor Science Publishers Inc., Ann Arbor MI, 1978

III. **Apparatus/Reagents:**

Compound Microscope

IV. **Safety Precautions:**

V. **Procedures:**

Basic Microscope Setup:

Turn on the light source and adjust the light intensity. Make sure the eyepiece with the micrometer is in the right eyepiece tube. Align the positioning pin on the eyepiece with the slot in the tube and insert Focus the right eyepiece with the micrometer to bring the eyepiece micrometer into focus by adjusting the knurled ring on the eyepiece. Sharply focus on the specimen. The specimen and the micrometer should both be in sharp focus through the right eye lens. Look into the left eyepiece and the left eye only, adjust the diopter adjustment ring on the left eyepiece to bring the specimen into the sharpest focus.

Stage Centering Procedure:

Place a specimen on the stage and focus on it with the 40X objective. Select a point on the specimen and locate it under the crosshairs. Note this as point A. Rotate the specimen 180 degrees and this will be point C. The center position is halfway between point A and point C. Use the centering wrench in the stage to move point C halfway to the cross hairs. Repeat steps 2-4 until the stage is centered as much as possible.

Objective Centering:

Place a specimen on the stage and focus on it with the 10X objective. Select a point on the specimen and locate it under the cross hairs. Call this point A. Rotate the specimen 180 degrees. Note the point on the specimen as point C. The center position is halfway between point A and point C. Use the centering wrench in the stage to move point "C" halfway to the crosshairs. Repeat steps 2-4 until each of the objectives is centered as much as possible.

Koehler Illumination:

Check the focus of a specimen with the 10X objective. If the 4X objective is utilized, swivel the top condenser lens out of the light path. Open the aperture diaphragm on the condenser lens wide open. Make sure the top condenser lens is in position. Close down the aperture lens of 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. Center the image of the field diaphragm by using the centering screws on the condenser. Gradually widen the field diaphragm ensuring that it remains centered. Open it to just outside the field of view. Adjust the aperture iris diaphragm to the numerical aperture of the objective lens by stopping it down. Focus on the specimen. Remove the right eyepiece. Adjust the diaphragm until it leaves about 70-80% of the field of view. This completes the Koehler illumination setup for the microscope.

Tension of the coarse focus knob can be adjusted by rotating the adjustment ring. After coarse focus has been made on a specimen, one can lock the lever to prevent the objective from making contact with the specimen. This does not affect the adjustment of the fine focus.

VI. Quality Assurance/Quality Control:

See PPG for basic calibration and maintenance of stereomicroscopes.