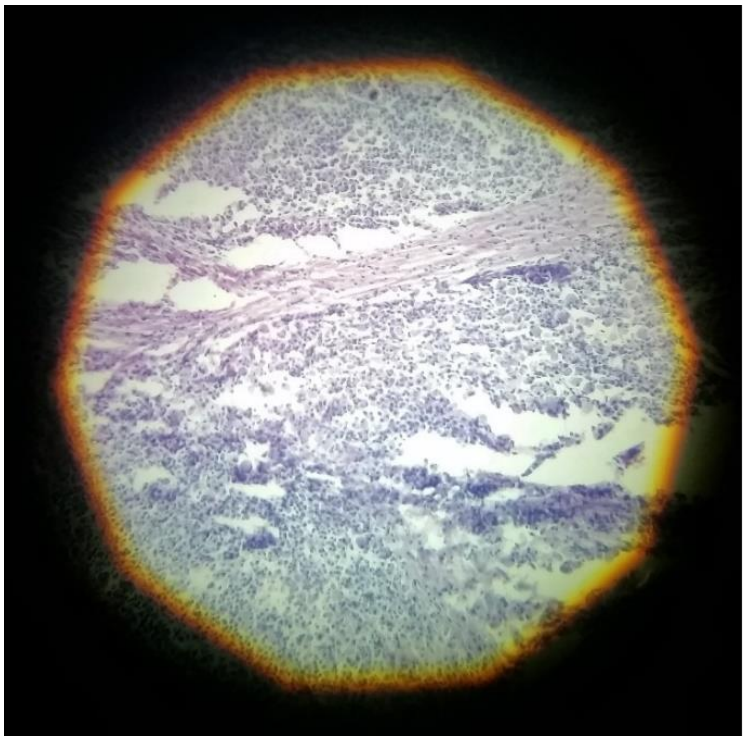
Köhler Illumination on the Olympus BH-2 Microscopes

Revision 2



Revision History		
Revision	**Description of Changes**	**Date**
1	Initial release	May 4, 2021
2	Title change	May 5, 2021

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Introduction

This document describes the implementation of Köhler Illumination, and the proper procedure to setup Köhler Illumination, on the various models of the Olympus BH-2 biological and polarizing microscopes.

Scope of this Document

The procedure described here was performed using an Olympus BHT microscope, but this procedure also applies to the BHS, BHSP, BHSU, BHTP, and BHTU models as well.

In the Days Before Köhler Illumination

An early, yet major development, in the evolution of microscopy was the technique known as Critical Illumination (also called *Source-Focused Illumination* or *Nelsonian Illumination*). This technique, which was originated by a British microscopist by the name of Edward Miles Nelson, used optical principles first introduced by Ernst Abbe, and was a huge improvement over non-focused lighting. Nelson's method utilized a sub-stage condenser to project a focused image of the light source directly onto the specimen under observation, thereby providing a much brighter visual field and higher resolving power than could be obtained using non-focused illumination. Critical Illumination was the standard illumination method of the late nineteenth century and is still used to this day in most microscopes intended for the educational and hobbyist markets. The primary drawback of Critical Illumination is that since the image of the light source is focused directly onto the specimen, any non-uniformity in the lighting source (such as in the filament of a halogen bulb or the emitter of an LED) is therefore directly visible to the observer as non-uniformity in the brightness of the visual field. Although this is not usually a problem for direct visual observations, such non-uniformity can be very noticeable in photomicrographic images. To be fair, in Nelson's day, either natural daylight or the flame of an oil or gas lamp would have been used as the source of illumination, and the uniformity of these sources was quite good, allowing for acceptable viewing and even acceptable photomicrography. It should be appreciated that much of the early and important work in the field of microscopy was performed on microscopes equipped for Critical Illumination. Eventually, electric lighting (whether tungsten, halogen, or later still, LED) came to be almost universally used, and these lighting sources exhibit significantly more non-uniformity than the flame and blue skies used in Nelson's day. Because of the inherent non-uniformity of electrical lighting sources, designers added a ground-glass diffuser between the light source and the substage condenser (or added an etched diffusion surface onto the lamp glass itself), which significantly improved the performance of these new electrically illuminated systems using Critical Illumination. However, the addition of the ground-glass diffusers introduced a new problem. Rather than focusing an image of the non-uniform illumination source onto the specimen plane, the condenser instead focused an image of the ground-glass diffuser onto the specimen plane, which appears as a distracting, grainy texture in the observed image. As a work-around to alleviate this problem on scopes equipped for Critical Illumination, the substage condenser is typically defocused a bit from the critical-focus point, by raising it slightly. This effectively obscures the graininess that would otherwise be visible to the observer.

Köhler Illumination

The lighting technique known as *Köhler Illumination* was first described by August Köhler in 1893. Köhler's method of illumination uses one or more converging lenses to form an image of the light source at the front aperture plane of the substage condenser, allowing light to emerge from the condenser as a parallel beam (i.e., with parallel rays) to provide perfectly defocused illumination of the visual field. Perfectly defocused illumination prevents any non-uniformity in the lighting source from appearing in the specimen plane. Köhler Illumination provides very even illumination when using non-uniform illumination sources, and because of this it is now found in virtually all upper-tier microscopes manufactured today.

The Evolution of Köhler Illumination

The original implementation of Köhler Illumination was rather complicated and somewhat fussy to setup. The light source/collection lens had to be placed the perfect distance from the microscope, in order to achieve the proper focus plane (i.e., focused at the front aperture plane of the substage condenser) with the focused image falling in the exact center of the aperture plane.

Pre-Focused Köhler: Eventually, microscope designers began to include the illumination source integral to the microscope stand itself, such that the fussiness of the lamp and collector lens placement was no longer an issue. By fixing these pieces into the stand at precise locations, everything was right where it needed to be in these pre-focused systems, and that little bit of fussiness was henceforth eliminated.

Pre-Centered Köhler: As nice as the pre-focused Köhler scopes were, they still needed a provision to mechanically center the lamp, since the manufacture of glass bulbs was such that the physical placement of the

filaments were not well controlled, and adjustments were needed to put the filament at exactly the correct location at the front aperture plane of the condenser. More recently, the manufacture of halogen incandescent lamps has progressed to the point where the physical placement of the filament within the glass envelope is well controlled, and centering adjustments for the lamp source are no longer necessary. This is even more true in LED-equipped scopes, where the placement of the LED emitter can be precisely controlled during manufacture. The convenience of these pre-centered Köhler scopes means they are here to stay.

Modified Köhler: The implementation of Köhler Illumination in microscopes made today does not conform to what some purists would call *true* Köhler Illumination, which is to say, Köhler Illumination as August Köhler originally described it. Rather, these modern scopes utilize what is sometimes referred to by purists as *modified* Köhler Illumination, where a ground-glass diffuser is present somewhere in the illumination path such that the image formed at the front aperture plane of the substage condenser is an image of the diffused light source, rather than an image of the raw halogen lamp filament.

The advantage of modified Köhler Illumination over true Köhler Illumination is that true Köhler relies on the source illuminator being completely planar, so that it can be properly focused onto the front aperture plane of the substage condenser. Although coming very close, real-world lighting sources do not meet this total-planarity ideal, hence some slight non-uniformity of illumination in the specimen plane subsequently occurs when true Köhler illumination is used with real-world lighting sources. The use of a ground-glass diffuser allows the diffused light source, which appears as a nearly ideal planar source, to be properly focused onto the front aperture plane of the substage condenser, thereby providing a more uniformly illuminated specimen than could be achieved without the diffuser. Some intensity loss invariably results from the scattering of light by the diffuser, but the resulting improvement in lighting uniformity is generally worth the tradeoff of brightness.

This diffuser was not present in Köhler's original description, since the diffuser would have prevented the operator from properly centering the filament image. Some Köhler Illumination setups include a spot for a removable diffusion filter, such that the filter can be removed to allow the operator to center the filament, then replaced to provide more uniform illumination during observations.

Köhler Illumination in the BH-2 Scopes

Olympus BH-2 microscopes (and most other modern Köhler-equipped microscopes) utilize pre-centered, pre-focused, modified Köhler Illumination, as described above. In the BH-2, the collector lens and field diaphragm are integral to the microscope base, as well as a mirror and exit lens to provide an image of the light source at the front aperture plane of the condenser. The lamphouse with its pre-centered halogen lamp is attached to the rear of the microscope base.

Setting Up Köhler Illumination on the BH-2

The following procedure describes the setup of Köhler Illumination on the Olympus BH-2 microscopes.

Set the Phase Condenser to the Brightfield Setting

This step applies only if your microscope is equipped with a BH2-PC or BH2-PCD Zernike-style phase contrast condenser (see **Figure 1** and **Figure 2**). If your scope is not equipped with a phase contrast condenser, skip ahead to the next step.

Figure 1 – Top view of BH2-PC/PCD condenser

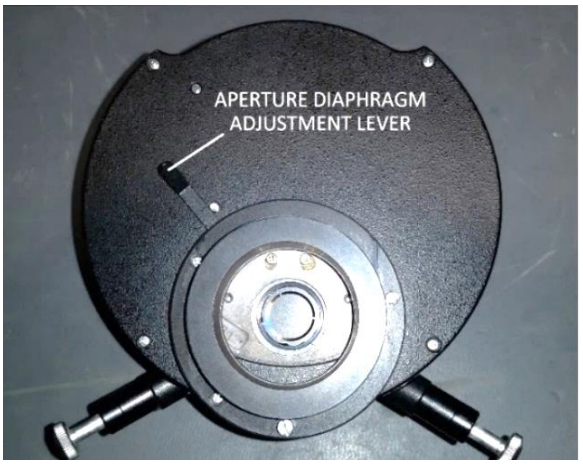

Figure 2 – Bottom view of BH2-PC/PCD condenser

PHASE CONDENSER ONLY: Rotate the selector dial on the front of the phase contrast condenser until the number "0" is visible on the front of the dial and the mechanical detent clicks in on this setting. This is the correct condenser setting for brightfield observations (see **Figure 3**).

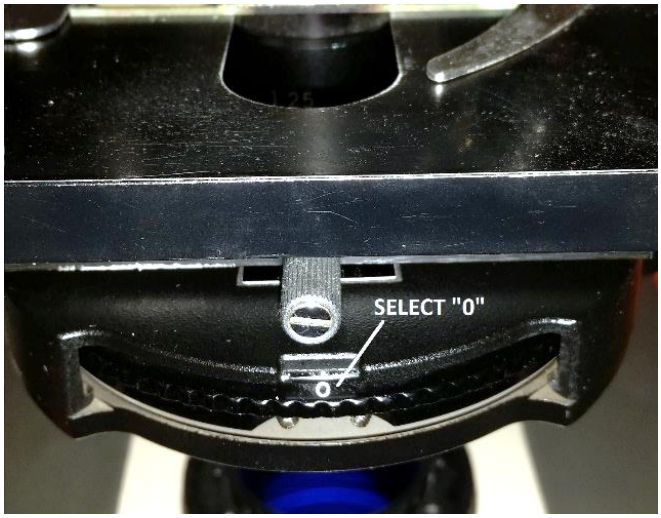

Figure 3 – Set selector dial for brightfield viewing

Focus on Specimen Slide with Desired Objective

Set the knurled field-adjustment ring (see **Figure 6**) to the fully clockwise setting Select the lowest-power objective available on the microscope. Place a suitable specimen slide onto the stage and adjust the specimen positioning, lighting intensity, focusing, and condenser aperture setting (see **Figure 2** if you have a phase contrast condenser and **Figure 4** if you have a standard Abbe condenser) so that the specimen detail is clearly visible through the eyepieces. Following the usual sequence, work your way up from the lowest-power objective to the objective that you wish to set up for Köhler illumination, repeating the above adjustments as necessary as you proceed through the various objectives (see **Figure 5**).

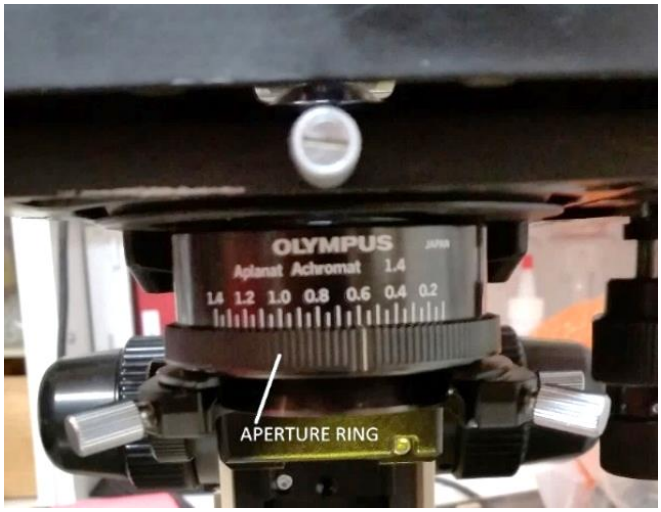

Figure 4 – Aperture ring on the BH2-CD condenser

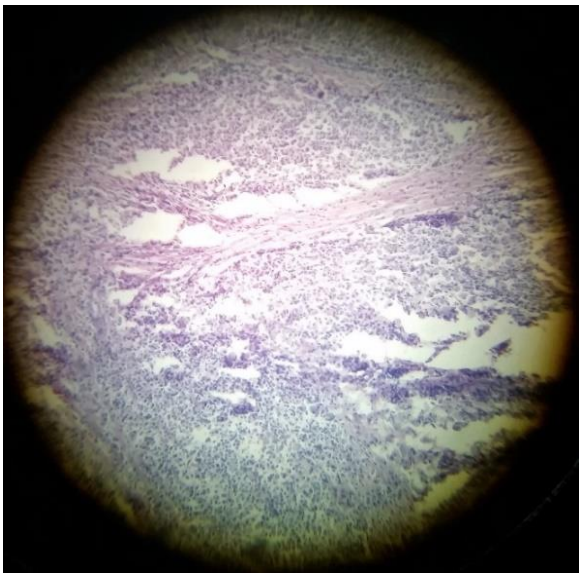

Figure 5 – Specimen properly focused for viewing

Partially Close the Field Diaphragm

With the specimen in sharp focus using the desired objective, partially close the field aperture diaphragm by turning the knurled control ring counterclockwise until a reduced spot of illumination is visible through the eyepieces (see **Figure 6** and **Figure 7**).

Figure 6 – Adjust the field diaphragm control ring

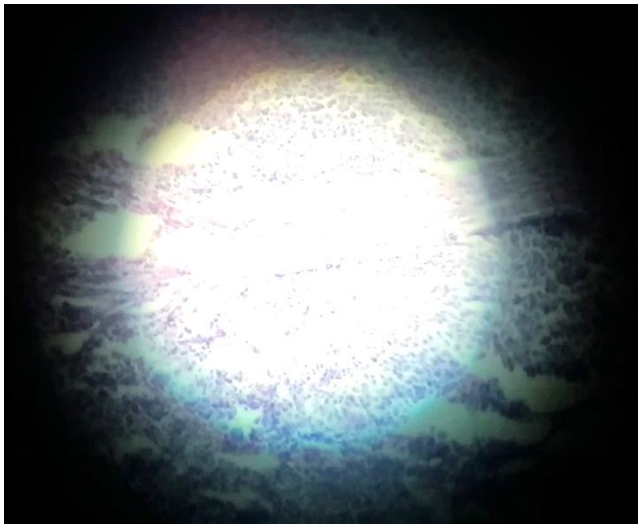

Figure 7 – The view with field diaphragm partially closed

Focus the Substage Condenser

Use the condenser focus knob to adjust the vertical position of the substage condenser, (see **Figure 8**), until the outer perimeter of the illuminated spot seen in the visual field is sharply focused (see **Figure 9**). This illuminated spot is the image of the field diaphragm aperture. Note that most all of the BH-2 condenser types, except for the BH2-AAC condenser, are poorly corrected for lateral chromatic aberration, and in these cases you will see color fringing in the image of the field diaphragm aperture, on both sides of the critical focus point. If you experience this, just pick the intermediate position between equal amounts of red and blue fringing and go with that.

Figure 8 – Focus spot using the condenser focus knob

Figure 9 – The illuminated spot properly focused

Center the Substage Condenser

Next, rotate the knurled field diaphragm control ring (see **Figure 6**), clockwise to increase the size of the illuminated spot, while also using the two orthogonal centering thumbscrews on the condenser yoke (see **Figure 10**), to adjust the centering of the light spot. Repeat this as necessary until the illuminated spot nearly fills the entire field of view through the eyepieces and is well centered within the visual field (see **Figure 11**).

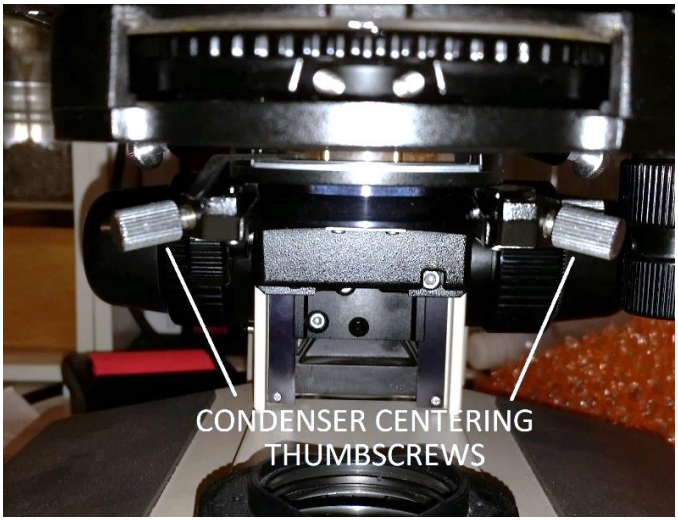

Figure 10 – The condenser centering thumbscrews

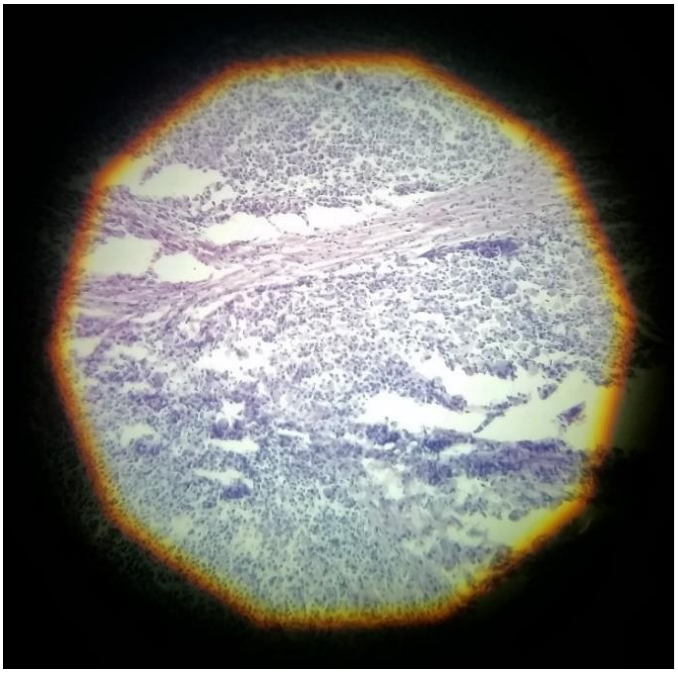

Figure 11 – Illuminated spot properly centered

Adjust Field Diaphragm to Fill the Field of View

With the image of the field diaphragm aperture properly focused and centered within the field of view, rotate the knurled field diaphragm control ring (see **Figure 6**) clockwise to increase the size of the illuminated spot such that the edge of the spot moves just beyond the borders of the visual field and can no longer be seen. If necessary, tweak the centering of the condenser with the two centering thumbscrews (see **Figure 10**) so that the illuminated spot remains centered as you make this final adjustment of the field diaphragm. At this point you should see the entire field of view with no evidence of the field diaphragm leaves

at all (see **Figure 12**). The microscope is now properly configured for Köhler illumination using this particular objective.

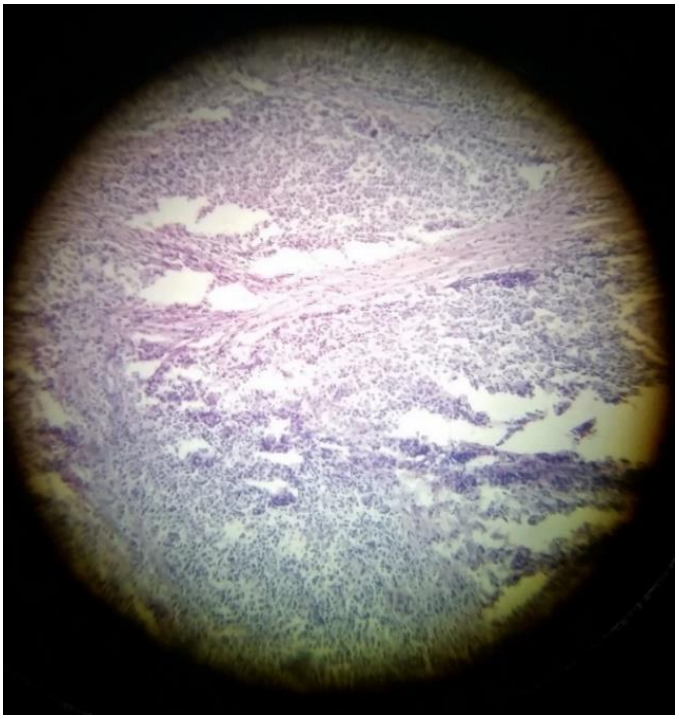

Figure 12 – Properly centered and filling the view

Repeat for Each Objective Change

Note that the Köhler setup procedure described above must be repeated every time the objective is changed.

Is this Really Worth the Effort?

Is it really worth the effort to do all of this? For routing viewing, perhaps it is not worth the effort to do a Köhler setup every time the objective is changed. Certainly, do not bother to do this as you progress through the objectives to get to the objective you wish to use. But once you get to the one that you do plan to use, now is the time to properly setup for Köhler Illumination. With a bit of practice, it takes only a few seconds to do this, and it soon becomes automatic. If you intend to take any photos of the specimen, then by all means take the time to do a proper Köhler setup first, as without this you cannot get the best field uniformity and image contrast that your scope is capable of providing.

How to Contact the Author

Please feel free to direct any questions or comments regarding this document (or BH-2 microscopes in general) to the author as listed on the cover page of this document.