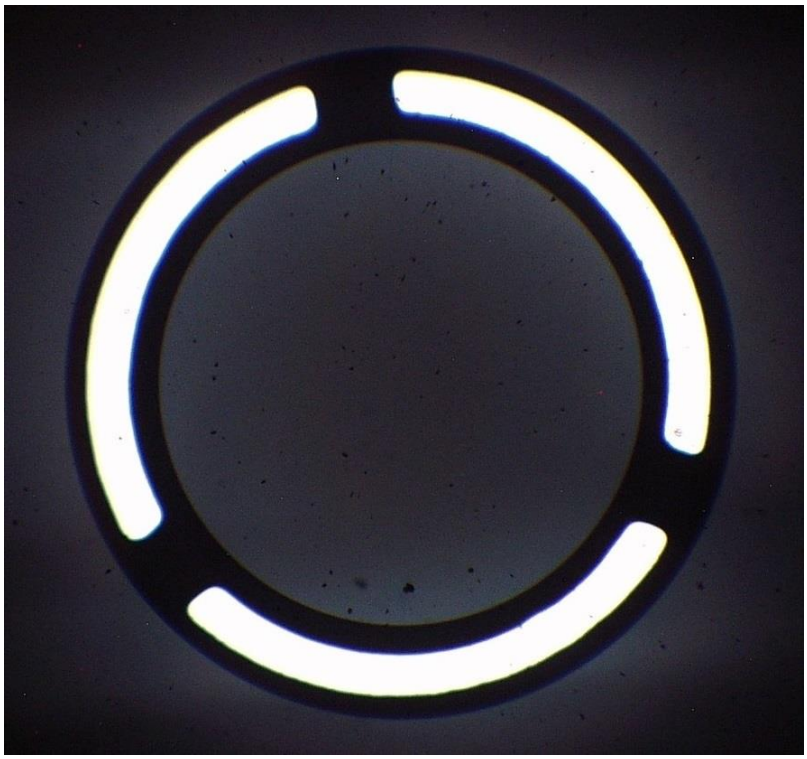
Phase Contrast on the Olympus BH-2 Microscopes

Revision 1



Revision History		
Revision	**Description of Changes**	**Date**
1	Initial release	May 5, 2021

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Introduction

This document describes the proper procedures to set up an Olympus BH-2 microscope for phase contrast viewing.

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.

What is Phase Contrast?

The technique of phase contrast microscopy was developed in the 1930s by Dutch physicist Frits Zernike and began to be broadly used by 1942. Zernike was awarded the Nobel Prize in Physics for his achievement in 1953. Phase contrast techniques are most useful for studying living, non-stained specimens, since live specimens cannot typically be stained without affecting their behavior or killing them outright. For these types of unstained specimens, phase contrast provides significantly increased contrast as compared to conventional brightfield microscopy. In a nutshell, phase contrast optics exaggerate the differences in the phase relationships between the light waves in the background illumination and the light waves passing through the specimen, so that they can constructively or de-constructively interfere with each other at the intermediate image plane, thereby converting invisible phase differences into visible image contrast.

How Does Phase Contrast Work?

In conventional brightfield microscopy, a visible image is formed by wave interference at the intermediate image plane of the background illumination (the background illumination is the light that does not pass through the specimen, which is also known as the "S", or *surround* wave-front) and of the diffracted light (the diffracted light is the light that passes through the specimen under observation, which is also known as the "D", or *diffracted* wave-front). The image of the specimen is visible to the observer due to the diffraction, absorption, and phase-shifting that occurs as the D wave-front passes through the specimen under observation. Diffraction in the D wave-front occurs as a result of detail in the specimen. Absorption occurs as a result of the specimen being not completely transparent. Phase shift occurs as a result of differences in the refractive index of the specimen, as compared to the surrounding medium.

When viewing live, unstained specimens in brightfield, the specimen is often difficult to see since light absorption can be minimal and since the constructive/destructive interference that occurs as a result of the phase-shifted D wave-front is minimal as well. Phase contrast microscopy utilizes special optics (both in the condenser and in the objectives) to accomplish two things: 1) The S wave-front (i.e., the background illumination) is decreased in amplitude by the phase ring in the objective so that the intensity of the D wave-front will not be swamped by the otherwise bright background lighting. 2) The S wave-front is phase shifted by a quarter wavelength by the phase ring in the objective, thereby exaggerating the constructive or destructive interference that occurs between the S and D wave-fronts at the intermediate image plane. The result of these two things is that images of live, unstained specimens have significantly higher contrast than could otherwise be obtained using convention brightfield microscopy.

Phase Contrast Types

Olympus phase contrast optics for the BH-2 are available in two basic types: *Positive* and *Negative*, as indicated by the markings on the objective barrel. Positive phase contrast objectives are marked with a "P" (such as "PL" or "PLL") and negative objectives are marked with an "N" (such as "NH" or "NM").

In positive phase contrast, the phase ring in the objective advances the S wave-front by a quarter wavelength, relative to the D wave-front, whereas in negative phase contrast, the phase ring retards the S wave-front by a quarter wavelength, relative to the D wave-front. In both cases, the phase of the D wave-front is retarded by areas of the specimen which have a higher refractive index than the surrounding medium, and advanced by the areas of the specimen which have a lower refractive index than the surrounding medium.

In positive phase contrast, the advanced S wave and the retarded D wave destructively interfere at the intermediate image plane, resulting in the areas of the specimen with a higher refractive index than the surrounding medium appearing darker than the neutral-gray background. In negative phase contrast, the exact opposite occurs. The retarded S wave and the retarded D wave constructively interfere at the intermediate image plane, resulting in the areas of the specimen with a higher refractive index than the surrounding medium appearing lighter than the neutral-gray background.

Setting Up for Phase Contrast Microscopy

The following equipment will be needed to utilize phase contrast on Olympus BH-2 microscopes.

- Olympus BH-2 microscope
- BH2-PC or BH2-PCD Zernike-style phase contrast condenser
- Olympus CT-5 or CT-30 phase-centering telescope (as appropriate)

- Olympus IF-550 (interference) or 45G533 (absorptive) filter
- One or more Olympus LB phase contrast objectives

Match Selector Dial with the Low-Power Objective

Select the lowest-powered phase contrast objective available on your microscope. Rotate the selector dial on the front of the phase contrast condenser until the number visible on the front of the dial matches the selected phase contrast objective (see **Figure 1**). Make sure the selected number on the dial clicks in place.

Figure 1 – Set condenser dial to match the objective

Replace Eyepiece with Phase-Centering Telescope

Carefully remove the eyepiece from the right-hand ocular tube and insert an Olympus phase-centering telescope[1] in its place (see **Figure 2**).

Figure 2 – Replace right eyepiece with phase telescope

Focus the Phase-Centering Telescope

Look into the phase-centering telescope. With the lighting intensity set to a comfortable viewing level, you should see a bright ring of light and also a darker ring, similar to that shown in **Figure 3**. Note that the image may be so blurry that these features cannot be readily discerned at this point. Also note that the relative positioning of the two rings may vary from that shown in **Figure 3**.

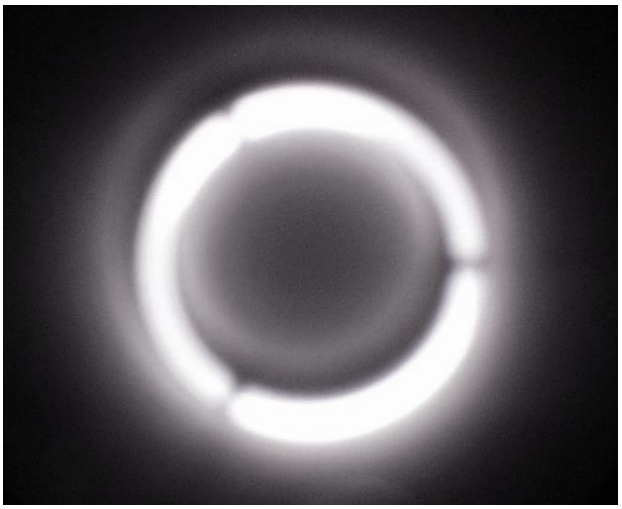

Figure 3 – Phase telescope improperly focused

Rotate the knurled focusing ring on the phase-centering telescope (see **Figure 4**) until the image seen through the phase-centering telescope is sharply focused (see **Figure 5**).

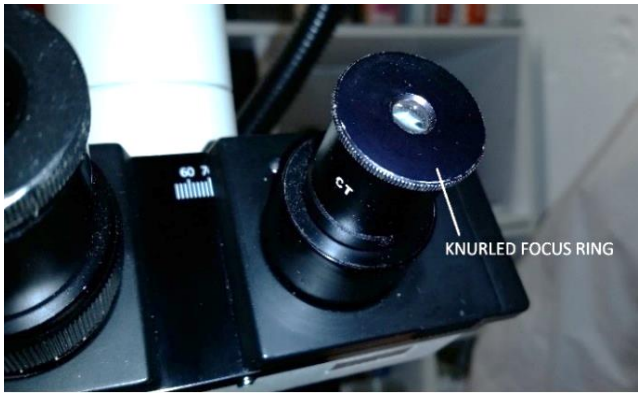

Figure 4 – Adjust knurled focus ring to focus the image

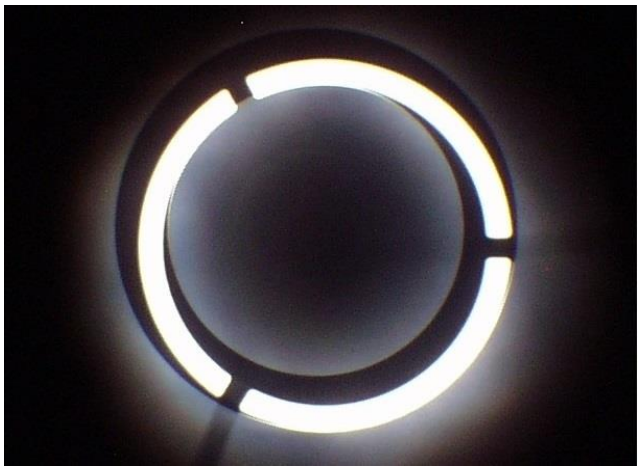

Figure 5 – The phase telescope is properly focused

[1] Use the "CT-5" for 23mm tubes and use the "CT-30" for 30mm tubes. Note that other manufacturer's centering telescopes may be substituted for the Olympus CT, so long as the barrel diameter of the phase telescope is compatible with your ocular tubes.

Center the Selected Phase Annulus

Per the following procedure, carefully adjust the two orthogonal, spring-loaded centering thumbscrews on the rear of the phase contrast condenser (see **Figure 6**) in order to center the bright image of the phase annulus within the dark image of the phase ring in the objective (see **Figure 7** and **Figure 8**).

Figure 6 – Centering screws of BH2-PC/PCD condenser

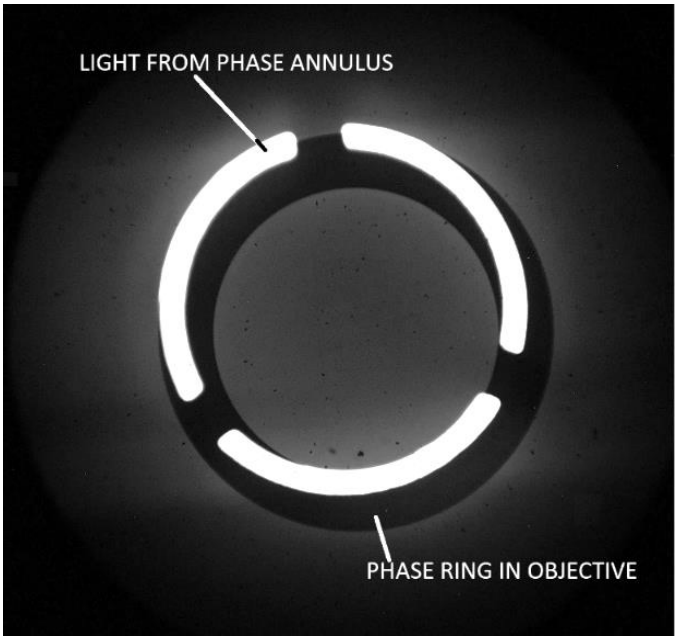

Figure 7 – The phase ring and phase annulus images

To adjust the position of the phase annulus, carefully depress both of the spring-loaded centering thumbscrews on the rear of the phase contrast condenser (see **Figure 6**) until the thumbscrews engage with the internal centering screws for the selected phase annulus. Then, while watching through the phase-centering telescope, adjust the thumbscrews to get a feel for how the centering adjustments work and how the two thumbscrews interact with each other. Once you have gained a feel for it, use the thumbscrews to adjust the position of the selected phase annulus so that its image (the bright ring) falls within the image of the phase ring in the objective (the dark ring) as shown in **Figure 8**.

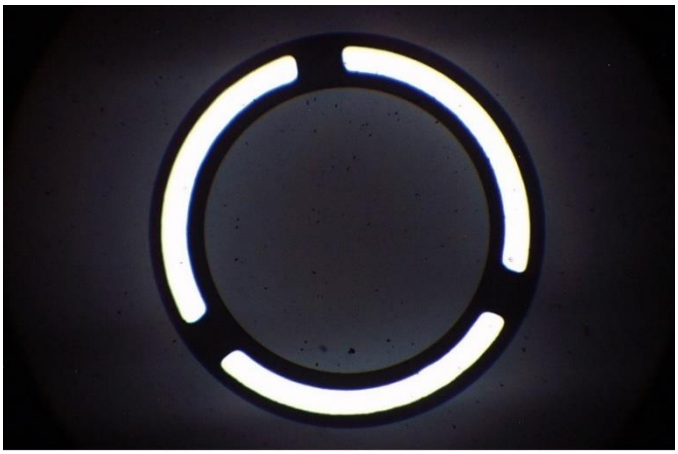

Figure 8 – The phase annulus properly centered

After the selected phase annulus has been properly centered, release the two centering thumbscrews and allow them to disengage from the internal centering screws for the phase annulus.

Caution: Never turn the thumbscrews counterclockwise beyond the point where the phase annulus ceases to move in the field of view, or clockwise beyond the point where their rotation begins to feel firm, and do not rotate the selector dial on the phase contrast condenser while one or both centering thumbscrews are engaged with the internal centering screws, otherwise damage to the phase contrast condenser may result.

Center the Remaining Phase Annuli

Repeat the centering procedure described above to center the phase annuli in the condenser for the remaining phase contrast objectives on the microscope.

Replace Centering Telescope with the Eyepiece

Once the various phase annuli have been properly centered, remove the phase-centering telescope from the right-hand ocular tube and replace it with the eyepiece.

Place Green/Yellow Filter into Filter Receptacle

Carefully place the green/yellow phase contrast filter (the IF-550 interference filter provides the best results, but lacking this, the 45G533 absorptive filter may be used instead) into the 45mm filter receptacle beneath the stage (see **Figure 9**).

Figure 9 – Place green/yellow filter into filter receptacle

Congratulations, you have now properly setup your BH-2 microscope for phase contrast viewing.

Using Phase Contrast

The hard part is now done. From here on out, to use phase contrast, just be sure to always match the number on the selector dial on the front of the phase contrast condenser with the magnification of the selected phase contrast objective. That is basically all there is to it. Don't forget to return the selector dial on the phase contrast condenser to the "0" setting whenever you wish to switch back to regular brightfield viewing.

Periodically Verify Centering of the Phase Annuli

It is good practice to re-check the centering of the phase annuli before starting a phase contrast observation session. With practice, you will come to see that once the phase annuli have been centered, they rarely need to be re-adjusted.

About the Phase Contrast Filter

Note that it is necessary to use the green/yellow filter in the illumination system, in order to provide light of the proper wavelengths with which the phase contrast objectives were designed to operate. This is critical if you wish to obtain optimal phase contrast results. This filter is particularly important when you wish to perform photomicrography, since that's when image quality is most critical. The inevitable green/yellow cast that the filter imparts may be removed from the images by converting them to grayscale.

For routine viewing, however, you may find that you prefer to omit the green filter from the illumination system, knowing that the phase contrast effects will be less than ideal, but the resulting color rendition will be much closer to normal.

Phase Contrast Artifacts

Certain visual artifacts will inevitably appear when viewing specimens under phase contrast, which can sometimes make it difficult to accurately interpret the resulting images. The bad news is you really cannot get rid of these artifacts. You will find that certain specimens are ideally suited for phase contrast, whereas others are better suited to regular brightfield viewing, because of these artifacts.

Halos: The most obvious phase contrast artifacts are the halos which you will see surrounding your specimens. These halos are caused by some of the diffracted light from the specimen passing through the phase ring in the objective. Ideally, only the background illumination should pass through the phase ring, and only diffracted light should pass through the areas inside and outside the phase ring. In positive phase contrast imaging (i.e., when using PL or PLL phase contrast objectives), this effect causes larger objects have a brighter edge, whereas in negative phase contrast (i.e., when using NM or NH phase contrast objectives), this effect causes them to have a darker edge.

Shade-Off Effect: Another phase contrast artifact is known as the *Shade-Off Effect*. In this case, the homogeneous parts of the image show up at the same brightness level as the background (i.e., the same as the surrounding medium). This occurs because although the light experiences a phase shift as it passes through these regions of the specimen, only minimal diffraction occurs and the angle it scatters is therefore limited, causing it to pass through the phase ring and therefore not experience the desired interference.

Contrast Inversion: A third phase contrast artifact is known as *Contrast Inversion*. In the case of positive phase contrast, objects with a high index of refraction situated next to objects with a low index of refraction will appear brighter than the background, instead of darker. This happens because in these cases the phase shift is not the usual quarter wave that should occur, and instead of the expected destructive interference occurring, constructive interference occurs instead. The opposite of this is true for negative phase contrast.

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.