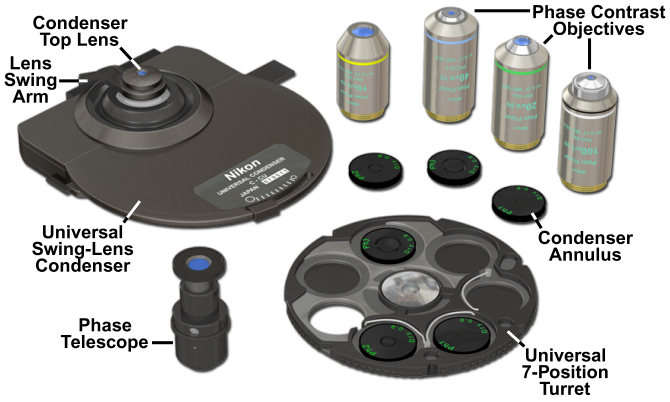
Phase Contrast Microscope Configuration

Phase contrast optical components can be added to virtually any brightfield microscope, provided the specialized phase objectives conform to the tube length parameters, and the condenser will accept an annular phase ring of the correct size. The major manufacturers all provide phase contrast accessories for their research and teaching-level microscopes, in both upright and inverted (tissue culture) configurations.

Figure 1 - Phase Contrast Optical Components



Typical phase contrast components available for the upright Nikon research microscopes from the Eclipse series are illustrated in Figure 1, although similar accessories are also produced by other manufacturers. The condenser presented in Figure 1 is a **universal** system designed for applications utilizing a wide range of magnifications (between 2x and 100x) and accessories for several contrast-enhancement techniques, including differential interference contrast (**DIC**), darkfield, and phase contrast. Objectives containing internal phase plates are offered with a variety of optical correction factors, ranging from simple achromats to plan apochromats. In addition, phase plates are available with several levels of surround wavefront attenuation to yield varying degrees of contrast and background intensity. Microscope objective lens manufacturers such as Nikon typically produce both positive and negative phase lenses.

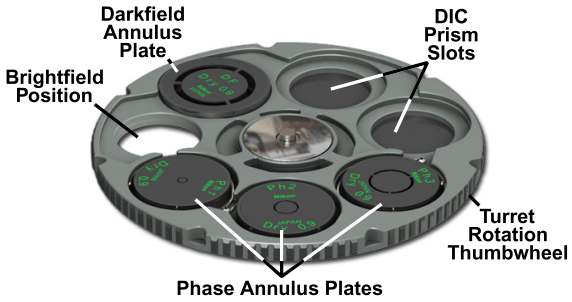
In order to align the condenser annulus with the phase plate in the objective rear focal plane, a phase telescope (as illustrated in Figure 1) that inserts into one of the microscope eyepiece observation tubes can be employed. A complete phase accessory kit ranges in cost from several hundred to several thousand dollars, depending upon the objective correction factor, complexity and optical correction of the substage condenser, and whether or not a phase telescope is included. The following sections describe these components in greater detail and present a guide to configuration and alignment of phase contrast microscopes.

Condenser Annulus Design

The phase contrast annuli (***annulus*** is the Latin term for **ring**, with the plural being ***annuli***) utilized in the substage condenser on an upright microscope, or within the condenser turret on the illumination pillar of an inverted microscope, must be specifically matched to a particular objective equipped with a corresponding phase plate. For example, a 20x objective and a 100x objective, both of which contain a phase plate near the diffraction plane (rear focal plane), will require condenser annuli having different diameters that correspond to the objective magnification and numerical aperture. By matching the condenser annulus to the objective phase plate, the microscope can be aligned to superimpose illuminating light rays passed through the annulus onto the objective phase ring to achieve phase contrast illumination.

A majority of the popular universal condenser systems designed for phase contrast microscopy are equipped with three or more removable annular diaphragms, which are available for use with 4x, 10x, 20x, 40x, 60x, and 100x objectives containing the appropriate phase plates. The **Ph1** (or equivalent) condenser annulus, which contains the smallest aperture, is designed to be employed with the lower power 10x and 20x objectives. Intermediate magnification objectives (40x and 60x) utilize the **Ph2** annulus, while the highest power (and numerical aperture) 100x objectives require the **Ph3** annulus, which contains the largest aperture. A specialized condenser annulus (**PhL**) is utilized with low-magnification objectives (4x and 5x) when the condenser swing-lens is removed from the optical pathway or in long working distance condensers designed for inverted tissue culture microscopes.

Figure 2 - Universal Condenser Turret Configuration



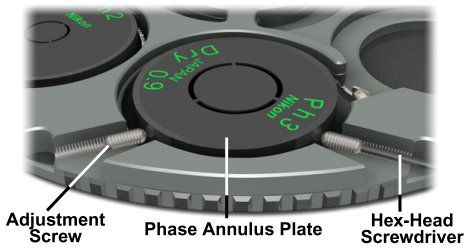
Condenser annulus inserts are circular aluminum plates having a stamped ring of varying dimensions in the center (as illustrated in **Figures 1 and 2**). After the stamping operation, annular disk units are anodized and dyed with a flat-black pigment to absorb stray light and ensure that illuminating light rays passing through the annulus follow a defined pathway. The central light stop, which varies in size depending upon the target objective aperture and magnification range, is positioned in the center of the plate and secured by three slim tabs spaced at 120-degree intervals. The annulus plate is either pressed or glued into a circular frame (also anodized and dyed black), which is fitted into a round opening in the condenser turret.

Modern universal condenser system turrets (see **Figure 2**) usually contain between five and eight open slot positions that can be fitted with annular phase contrast plates, DIC Nomarski (Wollaston) prism plates, or darkfield light stop plates. The basic construction of these plates is similar. Darkfield light stops are manufactured in a manner similar to phase annular diaphragms, but the annulus is generally wider and has a larger diameter to enable highly oblique light wavefronts to pass through. In contrast, DIC prisms are cut into circular slabs that are glued into an anodized frame and inserted into the turret.

The phase contrast condenser annular diaphragm plates are inserted into the appropriate position in the turret and secured into place with a spring clip that rides inside the periphery of the turret opening. Tension from the spring clip forces the annulus plate against a set of ad-

justment screws (or threaded pins) utilized for centering the annulus in the optical pathway (as illustrated in Figure 3). Several manufacturers offer condenser systems designed specifically for phase contrast that feature an integral annulus centering system. These condensers usually have a set of adjustment knobs that can be actuated by depressing the shaft of each knob until it locks into an annulus adjustment screw. After the screws are engaged, twisting the knobs will enable the operator to center the condenser annulus with respect to the objective phase plate.

Figure 3 *- Phase Condenser Annulus Plate Alignment*

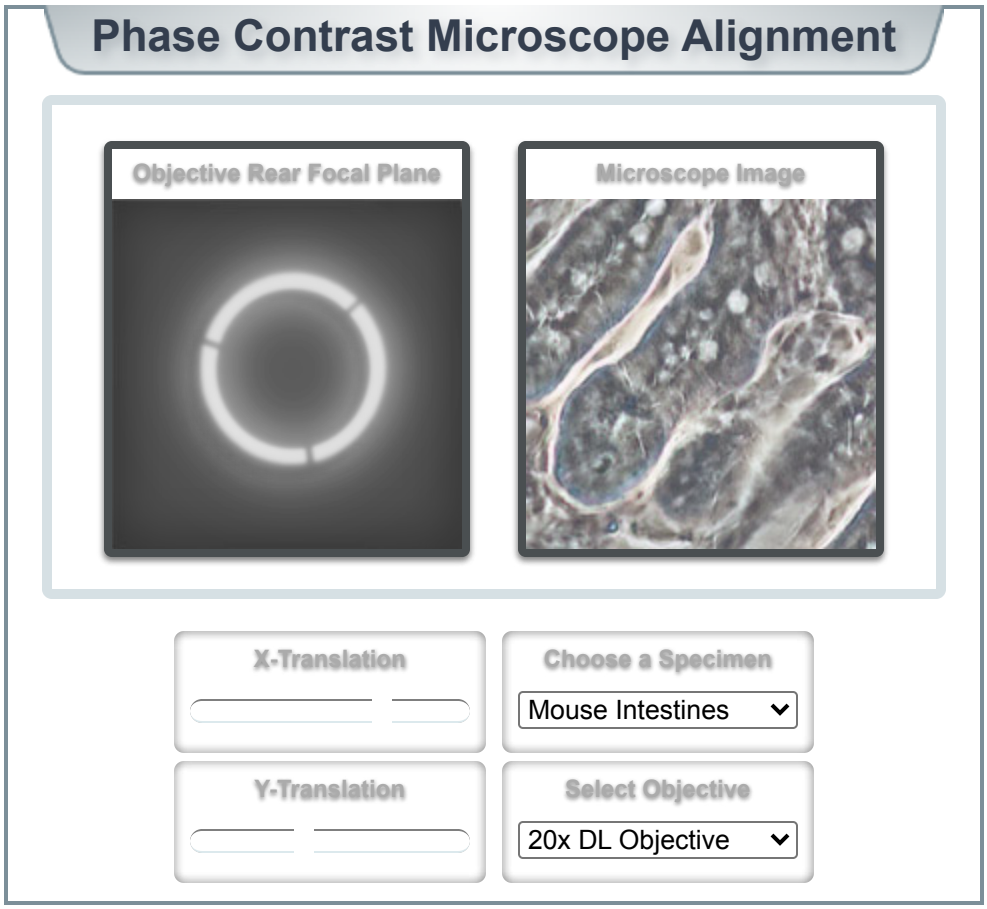


Whenever a universal microscope condenser is utilized for phase contrast observation, the operator should be certain to examine the position of the aperture diaphragm lever to ensure that the diaphragm is opened wider than the diameter of the condenser annulus. In fact, it is a good idea to always open the diaphragm to its widest position during phase contrast observations. Several manufacturers offer condensers designed exclusively for phase contrast, which have a spring-loaded mechanism that automatically opens the condenser aperture iris diaphragm when a phase annulus is rotated into the optical pathway, but disables this feature for brightfield observation with the same condenser. After concluding phase contrast experiments, the microscope can be returned to brightfield mode and the condenser iris diaphragm opening adjusted according to the objective numerical aperture.

Phase contrast condensers are available in a wide spectrum of optical correction levels, ranging from the basic Abbe design (no correction for chromatic or spherical aberration) to highly corrected aplanatic-achromatic systems that feature excellent performance. In addition, specialized phase contrast condensers are available with long working distance (**LWD**) optics, for both upright and inverted microscopes, in order to allow imaging of specimens contained in thick vessels. Many of the research-grade inverted microscopes have extra long working distance (**ELWD**) condensers with phase annuli that enable the observation of living cells in very large containers.

Interactive Tutorial - ***Phase Contrast Microscope Alignment***

Learn how to align a phase contrast microscope and examine variations in specimen appearance through the eyepieces (at different magnifications) when the condenser annulus is shifted into and out of alignment with the phase plate in the objective.



The specifications for phase contrast accessories can vary significantly from one manufacturer to another, and there is no accepted standard for phase annular diaphragm (and objective phase plate) dimensions with respect to either numerical aperture or magnification. As a result, the phase contrast objectives from one microscope manufacturer cannot usually be coupled to the phase condenser from another (and vice versa).

Single annulus phase condensers were very popular at one time, but have been largely supplanted by the universal turret models. The older condensers require removal and insertion of different phase annuli as the objective is changed, a cumbersome and time-consuming task that often must be repeated frequently when examining fine specimen detail at several magnifications. Centration of the annular diaphragm in a single annulus condenser is accomplished in a manner similar to that of the newer, universal condensers. Either flat-blade or Allen-type threaded pins or screws are utilized to press the annulus plate against a leaf or coil spring unit within the condenser housing.

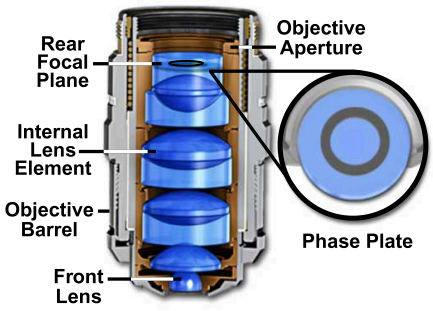
In order to adapt a standard Abbe, achromatic, or aplanatic substage condenser for phase contrast observations, a mechanism must be established for placing the annular diaphragm in or very near the condenser front focal (aperture) plane. Several condenser models are fabricated with the aperture iris diaphragm (always located in the focal plane) positioned at the base of the condenser, adjacent to slots designed to accept phase plates or darkfield light stops. Other condensers have the aperture plane located in the central region of the housing, which is relatively inaccessible to auxiliary components, such as a phase annulus.

Phase Contrast Objectives

The most important attribute of objectives designed for phase contrast microscopy is the presence of a specialized **phase plate** positioned in or very near to the diffraction or rear focal

plane. Phase plates are not interchangeable between objectives and are often permanently etched into one of the internal lens elements. The etched ring is coated with a partially absorbing metallic film that reduces light transmission, and is usually made so that the phase of light passing through will be advanced by one quarter-wavelength relative to light that is transmitted through the rest of the glass.

Figure 4 - Phase Contrast Objective

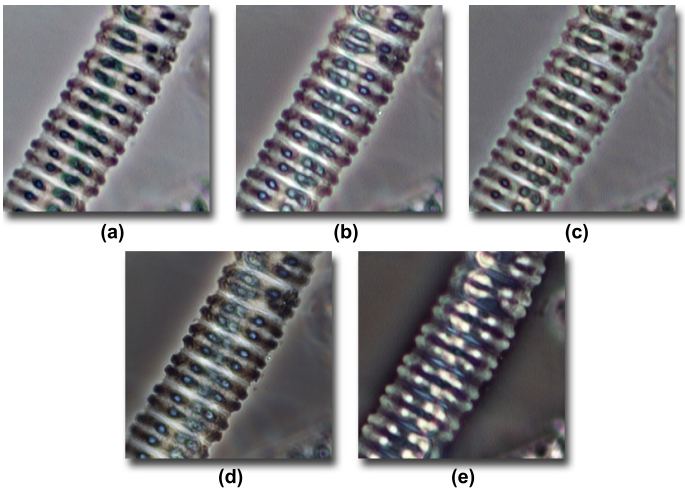


A cut-away diagram of a typical phase contrast objective is illustrated in **Figure 4**. The inset illustrates the phase plate that is positioned in the rear focal plane. Other portions of the objective, including most of the internal lens elements, are identical in construction to standard brightfield microscope objectives. The range of phase contrast objectives available from the major manufacturers covers almost the complete gamut of correction factors, including achromatic, plan achromatic, fluorite, and apochromatic. These objectives are also available with coverslip correction collars (for dry high numerical aperture objectives) and for immersion techniques in water, glycerin, and oil. In addition, phase contrast objectives are manufactured that vary in the neutral density and retardation value of the phase plate to produce a wide spectrum of contrast levels in both positive and negative phase contrast modes.

Nikon and other manufacturers produce a variety of phase contrast objectives (see **Figure 5**) that vary in sign (positive or negative), and range in contrast from relatively low to very high. In positive phase contrast, the most common form, optically dense portions of a thin specimen appear dark against a background having a lighter shade of gray. Negative phase contrast renders specimens bright, but superimposed over a dark background, similar to darkfield illumination.

Presented in **Figure 5** is a comparison of digital images recorded using the currently available Nikon phase contrast objectives to demonstrate the contrast variations afforded by changes in phase plate neutral density and surround wavefront retardation (or advancement) levels. The images are the same viewfield from a fixed and mounted preparation of ***Zygnema*** filamentous algae. **Figure 5(a), 5(b), and 5(c)** compare the dark low low, dark low and apodized phase contrast objectives (**DLL**, **DL**, and **ADL**, respectively), which produce similar degrees of contrast on a relatively light gray background. The dark and bright medium Nikon phase contrast objectives (**Figure 5(d) and 5(e)**) demonstrate positive and negative phase contrast on a medium gray background. Properties of the individual Nikon phase contrast objectives are discussed below.

Figure 5 - Nikon Phase Contrast Objectives



The degree of specimen contrast afforded by the various types of phase contrast objectives is complicated by the fact that large fluctuations in refractive index and thickness can produce contrast inversions and significant light scattering from specimen areas away from the focal plane. The density of the absorptive material applied to the phase plate (to attenuate the surround light wavefront) and the phase shifting properties (that retard or advance the surround wavefront) of the phase plates have a dramatic effect on the results observed in a phase contrast microscope. Several important features of the various phase contrast objectives produced by Nikon are listed below.

- **DL (Dark Low - Medium Contrast)** - DL objectives produce a dark image outline on a light gray background, and are the typical objectives utilized for all-purpose phase contrast observation. These objectives are designed to furnish the strongest dark contrast in specimens having a major difference in refractive index from that of the surrounding medium. The DL phase contrast objective is the most popular style for examination of cells and other semi-transparent living material and is especially suited for photomicrography and digital imaging.

- **DLL (Dark Low Low - Low Contrast)** - Similar in design to the DL objective, the DLL series yields better images in brightfield illumination and is often employed as a "universal" objective in microscope systems that utilize multiple illumination modes such as fluorescence, DIC, brightfield, and darkfield. The DLL phase contrast objective produces less contrast than the DL objective, but features higher light transmission values, optical correction, and numerical aperture than the standard DL counterpart. A majority of the DLL phase contrast objectives offered by the manufacturers have fluorite or apochromatic aberration correction levels.

- **ADL (Apodized Dark Low - Medium Contrast)** - Recently introduced by Nikon, the apodized phase contrast ADL objectives contain a secondary neutral density ring on either side of the central ring in the phase plate. Addition of the secondary rings assists in reducing unwanted "halo" effects often associated with imaging large particles or specimen features (such as nuclei, whole cells and fibers) in phase contrast microscopy. Apodized objectives are available in achromat optical correction and feature contrast levels similar to the DL objective series.

- **DM (Dark Medium - High Contrast)** - DM objectives produce a dark image outline on a medium gray background. These objectives are designed to be used for high image contrast with specimens having small phase shifts or refractive differences, such as fine fibers, flagella, cilia, granules, and very small particles. Usually restricted to higher magnification objectives having large numerical apertures (fluorites and

apochromats), DM phase contrast objectives perform well with very thin specimens, but often display a reversal of contrast when thick specimens are imaged.

- **BM (Bright Medium - High Negative Contrast)** - Often referred to as negative phase contrast, BM objectives produce a bright image outline on a medium gray background. BM objectives are ideal for visual examination of bacterial flagella, fibrin bundles, minute globules, and for blood cell counting.

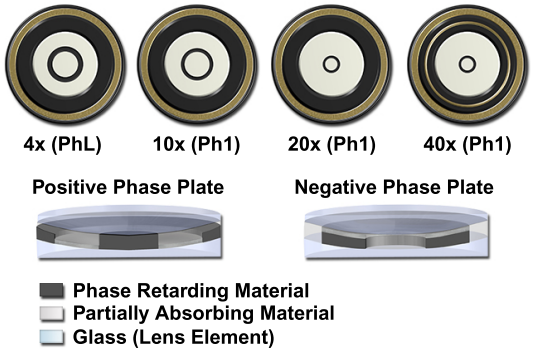
In order to enable the microscopist to quickly identify phase contrast objectives, many manufacturers inscribe important specifications, such as the magnification, numerical aperture, tube length correction, etc., on the outer barrel in green letters. The green alphanumeric color code serves to differentiate phase contrast objectives from ordinary brightfield, polarized, DIC, and fluorescence objectives which either use an alternative color code or the standard black lettering. In addition, phase contrast objectives have inscriptions on the barrel to indicate the objective is designed for phase contrast and also the matching annulus designation. A number of the most commonly encountered objective barrel engravings are described in Table 1. Phase contrast objectives can also be easily identified by holding the objective up to a bright light and peering through the glass to observe the centrally-positioned dark phase ring.

***Table 1** - Phase Contrast Objective Designations*

Abbreviation	Type
Phase, PHACO, PC	Phase Contrast
Ph 1, 2, 3, etc.	Phase Condenser Annulus 1, 2, 3, etc.
DL, DLL, DM, ADL	Dark Low, Dark Low Low, Dark Medium, Apodized Dark Low (Positive Phase Contrast)
PL, PLL, PM, PH	Positive Low, Positive Low Low, Positive Medium, Positive High Contrast (Positive Phase Contrast)
NL, NM, BM, NH	Negative Low, Negative Medium, Bright Medium, Negative High (Negative Phase Contrast)

A typical series of phase contrast objectives having increasing numerical aperture and magnification are presented in **Figure 6**. As a general rule, when objective numerical aperture and magnification is increased, the phase plate width and diameter both decrease (and the condenser annulus size increases). Also illustrated in **Figure 6** are cut-away diagrams showing the basic concepts behind positive and negative phase plate construction. The positive phase plate produces dark contrast and contains a partially absorbing film designed to reduce the amplitude of the surround wavefront. In addition, this plate contains phase retarding material designed to shift (retard) the phase of the diffracted light by 90 degrees. The negative phase plate also contains both phase retarding and partially absorbing materials. However, in this case, both materials are placed within the circular phase ring so that the undiffracted surround wavefront becomes the only species affected, and is attenuated and retarded in phase by 90 degrees.

Figure 6 - *Objective Apertures and Phase Contrast Optics*



Contrast is modulated in phase objectives by varying the properties of the phase plate, including the absorption of the metallic film (or anti-reflective coatings), the refractive index of the phase retarding material, and the thickness of the phase plate. Most of the phase plates available from manufacturers are produced by vacuum deposition of thin dielectric and metallic films onto a glass plate or directly onto one of the lens surfaces within the microscope objective. The role of the dielectric film is to shift the phase of light, while the metallic film attenuates undiffracted light intensity. Some manufacturers utilize multiple anti-reflective coatings in combination with the thin films to reduce the amount of glare and stray light reflected back into the optical system. If the phase plate is not formed on the surface of a lens, it is usually cemented between successive lenses that reside near the objective diffraction plane. The thickness and refractive indices of the dielectric, metallic, and anti-reflective films, as well as those of the optical cement, are carefully selected to produce the necessary phase shift between the complementary (diffraction) and conjugate (surround) areas of the phase plate. In optical terminology, phase plates that alter the phase of surround light relative to diffracted light by 90 degrees (either positive or negative) are termed **quarter wavelength** plates because of their effect on the optical path difference.

The Phase Telescope

If the condenser annulus, positioned in the front focal plane of the substage condenser, is not in exact alignment with the fixed phase plate in the objective (that is located centrally along the microscope optical axis), the contrast effect afforded by phase contrast optical systems will be dramatically compromised. In order to ensure proper microscope operation and to maximize the phase contrast effect, the objective rear focal plane should be examined with the condenser annulus in place to ensure that the microscope is properly aligned. This task can be accomplished using either a **phase telescope**, which can be inserted into one of the eyepiece observation tubes (in place of a normal eyepiece), or a **Bertrand lens** built into the microscope binocular (or trinocular) eyepiece tube assembly. The Nikon Ti2 inverted microscope features an internal Bertrand lens that allows for the phase ring to be either visualized by eye or directly on a camera for fine alignment, as well as an external phase contrast module for performing phase contrast imaging with objectives that don't have a phase annulus.

The phase telescope, also commonly referred to as an **auxiliary telescope** or **auxiliary microscope**, consists of a simple two or three lens telescope (illustrated in **Figure 7**) arranged to function as the objective and eyepiece combination of a miniature optical system that is intermediate between a telescope and microscope. The focal length of the phase telescope ranges from about 150 to 200 millimeters, enabling the device to focus on the objective rear focal plane when inserted into one of the microscope eyepiece observation tubes. The magnified view of the objective focal plane clearly reveals the spatial arrangement between the condenser annulus and objective phase ring when the phase telescope is properly focused. While

viewing the fixed phase ring in the objective, the operator can then perform adjustments to the condenser annulus position to align the two for phase contrast observation (see Figure 8).

Microscopes designed primarily for observation in polarized light or differential interference contrast often have a Bertrand lens that is incorporated into the eyepiece observation tube unit or an intermediate tube that is inserted between the eyepiece tube and the microscope body. The Bertrand lens is somewhat more sophisticated than a simple phase telescope, and acts as a relay lens, transferring an image of the objective focal plane to the microscope intermediate image plane located in the eyepiece aperture diaphragm. Thus, with a Bertrand lens inserted into the microscope optical pathway, the objective rear focal plane (and phase plate/condenser annulus alignment) can be observed through the microscope eyepieces. On most microscopes equipped with a Bertrand lens, the lens can be rotated into and out of the optical pathway by means of a small thumbwheel mechanism located beneath the eyepiece tubes. Later model microscopes often mount the Bertrand lens in a turret along with lenses that change the image magnification factor. Adjustment is made with a small knob that is labeled **B** or **Ph** for the Bertrand lens position, and **0** or some other number for the magnification lens.

Both the phase telescope and Bertrand lens must be equipped with a mechanism for adjustment of focus, because the location of the objective rear focal plane can vary with magnification (as well as a number of additional factors), and lie at different levels within the microscope. Focusing a typical phase telescope is accomplished simply by twisting the eye tube (see Figure 7) until the objective rear focal plane is sharply focused. In a similar manner, a majority of the Bertrand lenses in modern microscopes are equipped with a focusing thumbwheel that enables the operator to focus the Bertrand-lens-eyepiece combination on the phase plate in the objective. When utilizing a matched set of objectives (parfocal, with the same optical correction and tube length), the rear focal plane position should remain essentially the same as magnification is changed, thus reducing the necessity to refocus the phase telescope or Bertrand lens.

Phase Contrast Microscope Alignment

Before attempting to align a microscope for phase contrast observation, examine the instrument carefully to ensure that all objectives contain phase plates and are firmly seated in the nosepiece. The objectives should also be sequentially ordered in their arrangement on the nosepiece, from lower to higher magnification, in order to minimize changeover frequency between one condenser annulus and another. Often the 10x and 20x objectives share a common condenser annulus, as do the 40x and 60x objectives. Highly corrected objectives, such as apochromats designed for oil immersion, often have similar numerical apertures and utilize the same condenser annulus over a wider range of magnification (40x to 100x). Low power phase contrast (4x and 5x) usually requires a swing-lens condenser and a specialized condenser annulus.

The condenser annular plates should also be sequentially arranged, starting with the annulus designed for the lower magnification objectives and proceeding up to the one designed for the highest magnification. In general, the entire magnification range can be covered with three or four individual annuli. If a universal condenser is employed, position the lowest magnification annulus (for example, **PhL** or **Ph1**) to the right of the brightfield slot, and the other plates in sequential order in the neighboring slots. Often, the specimen must be examined in brightfield either before or after phase contrast observation, so this arrangement will provide an easy work flow.

Presented in Figure 8 are images of the objective rear focal plane in misalignment (**Figure 8(a) and 8(c)**) and after the condenser annulus and phase ring have been properly aligned (**Figure 8(e)**). Also illustrated in this figure are the corresponding images that appear when viewed through the eyepieces (**Figure 8(b), 8(d), and 8(f)**), which demonstrate how the speci-

men appears when the microscope is misaligned (Figure 8 **(b) and 8(d)**) and carefully aligned (Figure 8**(f)**) according to the procedure outlined below.

The following steps are recommended for the alignment of a phase contrast microscope.

- Place a brightly stained specimen on the stage and rotate the 10x phase contrast objective into the optical pathway in brightfield illumination mode. Focus the specimen, and close the field diaphragm until it enters the edges of the viewfield. Using the condenser height adjustment knob, position the substage condenser so the individual leaves of the field diaphragm are in sharp focus, and use the main condenser centering adjustment knobs to ensure the field diaphragm is centered in the field of view. Carefully review the microscope configuration to ensure that Köhler illumination has been achieved, and the specimen is in sharp focus.

- Remove the stained specimen and place a phase specimen on the microscope stage. In cases where the target specimen has only minimal optical path differences (and may be difficult to visualize), align the microscope with a specimen known to produce high contrast in phase contrast mode. Rotate the condenser turret until the appropriate annulus is positioned in the optical pathway (**Ph1** or the equivalent for the 10x objective). Check to ensure that the condenser annulus color code or inscription matches that of the objective. Examine the position of the condenser aperture diaphragm lever and move it to the widest iris position (condensers designed for phase contrast may do this automatically).

- If the microscope is equipped with a Bertrand lens, use the thumbwheel control to swing the lens into place. Alternatively, remove one of the microscope eyepieces and insert a phase telescope into the observation tube.

- While peering through the eyepieces or phase telescope, adjust the focus of the Bertrand lens or telescope focus eye tube until the phase plate in the objective is in sharp focus, and the overlap between the bright image of the condenser annulus and dark neutral density material in the phase plate is apparent. In many cases, the microscope will initially be out of alignment and the annulus image will not accurately overlay the neutral density material in the phase plate (as illustrated in Figure 1**(a)** and Figure 1**(c)**).

- Locate the condenser annulus centering pins (or screws) and adjust the position of the annulus with a pair of screwdrivers or the appropriate knobs until it is coincident with the objective phase plate (Figure 1**(e)**). Note: do not attempt to adjust the position of the condenser annulus with the main condenser centering knobs (usually located on the condenser mounting bracket attached to the microscope). This effort will probably not achieve the intended condenser annulus alignment and will definitely compromise Köhler illumination conditions that should have been previously established.

- When the condenser annulus is properly centered, the ring of light passing through the condenser will be attenuated by the neutral density material applied to the objective phase plate, reducing the intensity of the annulus image. Therefore, if the condenser annulus is improperly centered, a bright crescent edge will appear adjacent to the neutral density material in the objective phase plate (Figures 1(a) and 1(c)). If the image of the annulus does not fit within the dark circle of the phase plate, then either the condenser is out of focus (and Köhler illumination is not established), or the phase telescope (or Bertrand lens) is not focused on the objective rear focal plane. In some cases, even when the phase telescope (or Bertrand lens) and substage condenser are properly focused, the condenser annulus image is blurry and appears out of focus. This

may be caused by low frequency diffraction from the specimen. If this occurs, remove the specimen from the stage and proceed with alignment of the microscope.

- If the condenser annulus image is significantly different in size from that of the objective phase plate, check to see if the wrong annulus plate has been installed in the condenser (the most probable cause). Another possibility, if high numerical aperture oil immersion objectives are being employed, is that the condenser front lens element is designed to be immersed. In this case, it may be difficult (or impossible) to superimpose the condenser annulus on the phase plate without a drop of oil between the condenser lens and the microscope slide (and/or between the objective and the coverslip).

- When the condenser annulus and objective phase plate are in proper alignment, the image illustrated in **Figure 1(e)** should appear in the phase telescope or eyepiece with a Bertrand lens in place. At this point, the microscope is properly configured for observation of the specimen with phase contrast illumination.

- Replace the phase telescope with the eyepiece, or rotate the Bertrand lens out of the optical pathway, and examine the specimen. The background should appear a neutral gray in color (depending upon the neutral density of the objective phase plate) with the specimen visible in high contrast.

Once the microscope has been aligned for phase contrast, it will generally hold its centration for a considerable number of objective/annuli changes, but should be checked periodically to ensure proper alignment. If the microscope starts to slip out of alignment, the images appearing in the eyepieces (or on a computer monitor) will appear increasingly more like those observed with brightfield illumination.

Most of the microscope manufacturers provide a green interference or absorption filter with their auxiliary phase contrast kits, because the filter will produce monochromatic light having the same wavelength used for the original calibration of the objective phase plates. As a result, contrast is increased when the filter is inserted into the optical pathway (usually between the illumination condenser lens and the field diaphragm). A majority of the commercial phase plates are designed to produce phase shifts of a quarter wavelength in the green (550 nanometers) portion of the visible light spectrum. Theoretically, if white light is utilized instead of monochromatic light, extinction by interference will not be complete for all colors, and contrast will suffer. This limitation is particularly important if achromat objectives, which are corrected for chromatic aberration only in the green region, are utilized for phase contrast observation or image recording. However, with the current highly corrected fluorite and apochromatic objectives, the difference in contrast often is negligible, and therefore, insignificant.

The key to successful imaging with phase contrast illumination is to properly align the microscope, and to ensure that sufficiently thin specimens are spread evenly within the mounting medium on the microscope slide. Images made with exceedingly thick specimens often suffer from out-of-focus blur and contrast inversion artifacts that can be difficult to interpret. If the microscope is utilized for an extended period of time, occasionally check the objective rear focal plane to verify alignment of the condenser annulus with the phase plate in the objective.

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