ACCU-SCOPE®

3025 SERIES
PHASE CONTRAST MICROSCOPY
INSTRUCTIONS

PHASE ANNULI ARE PRE-CENTERED

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PHASE CONTRAST MICROSCOPY

The normal microscopic object is seen because it has regions of varying density. In normal brightfield illumination a completely transparent specimen is very difficult to observe in detail because all areas of the specimen are equally dense. Darkfield illumination displays border effects in completely transparent specimens due to edge scattering and diffraction of light. Polarized light is useful when transparent specimens have directional or crystalline properties.

Phase contrast microscopy is a type of illumination system to observe transparent media. This form of illumination is utilized extensively in the study of transparent living cells without the need for staining or fixing while being able to obtain good image contrast. The light from phase contrast illumination arrives at the user's eyes at ½ the normal wavelength. This light altering system produces a visible image of an otherwise invisible, transparent specimen.

The optical light path necessary for phase contrast is shown in Figure 1. A clear annulus in the focal plane of the condenser is imaged at infinity by the condenser and then re-imaged by the objective in its rear focal plane. The undiffracted light passes through this image. It is reduced in intensity and given a one-quarter wave phase shift by means of an annular phase pattern in the rear focal plane of the objective. These two changes in the undiffracted portion of the beam simulate the phase and intensity distribution which would be present in the objective focal plane if the specimen had density variations rather than refractive index variations. As a result, the image formed by the beam interfering with the diffracted beam simulates that of a specimen having density variations.

IMAGE FORMATION BY PHASE CONTRAST

An annular aperture in the diaphragm placed in the focal plane of the substage condenser controls the illumination of the specimen. The aperture is imaged by the condenser and objective at the rear focal plane or at the exit pupil of the objective. A phase shifting element, or phase plate, is placed in the image plane. Light passing through the phase altering pattern acquires a ¼ wave length advance over that diffracted by the object structure and passes through that region of the phase plate not covered by the altering pattern. The resultant interference effects of the two portions of light form the final image. Altered phase relations in the illumination rays, induced by otherwise invisible elements in the specimen, are translated into brightness differences by the phase altering plate.

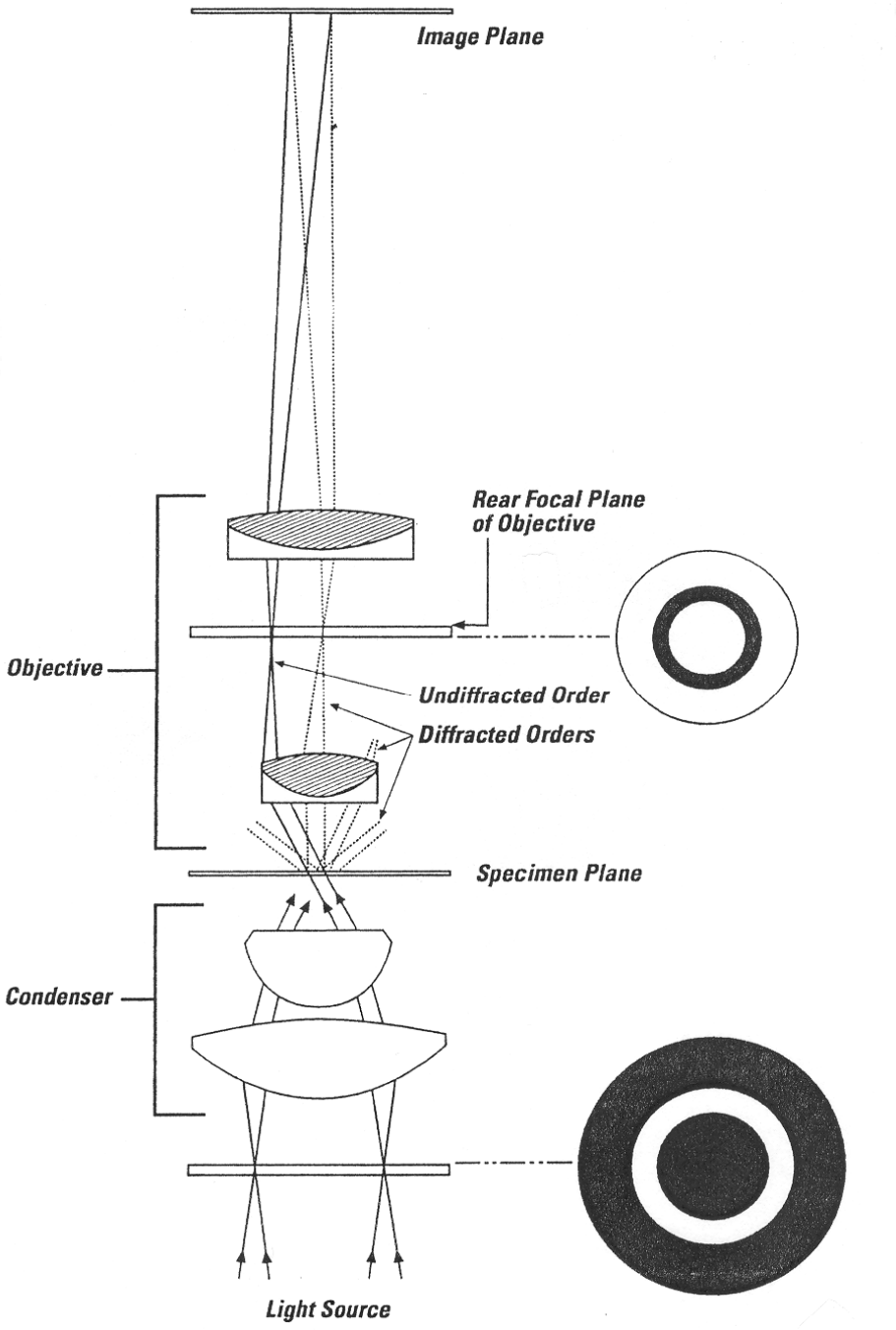
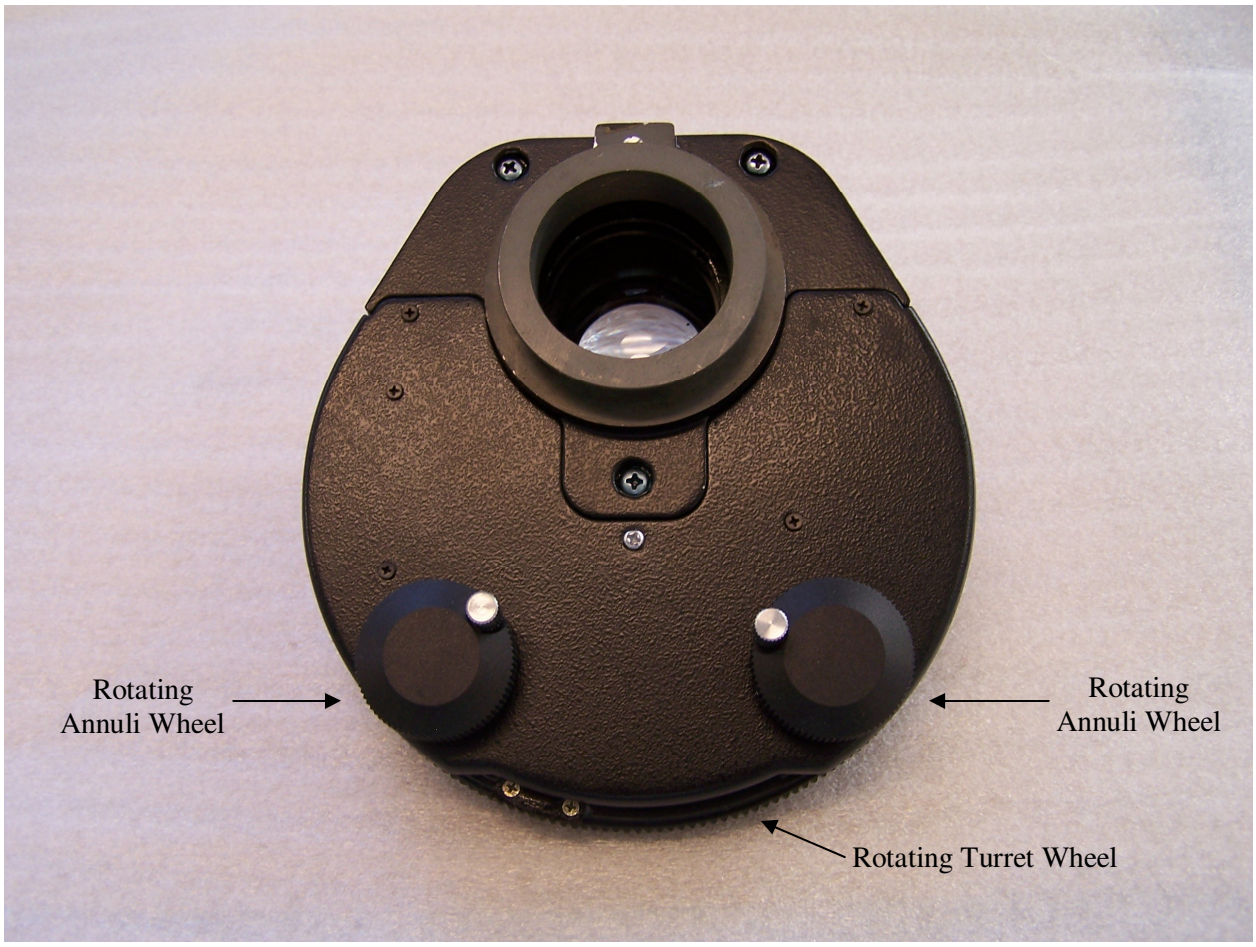


Image Plane
Rear Focal Plane
of Objective
Objective
Undiffracted Order
Diffracted Orders
Specimen Plane
Condenser
Light Source

Figure 1



3025 Series Turret Phase Condenser
(Bottom View)

Figure 2

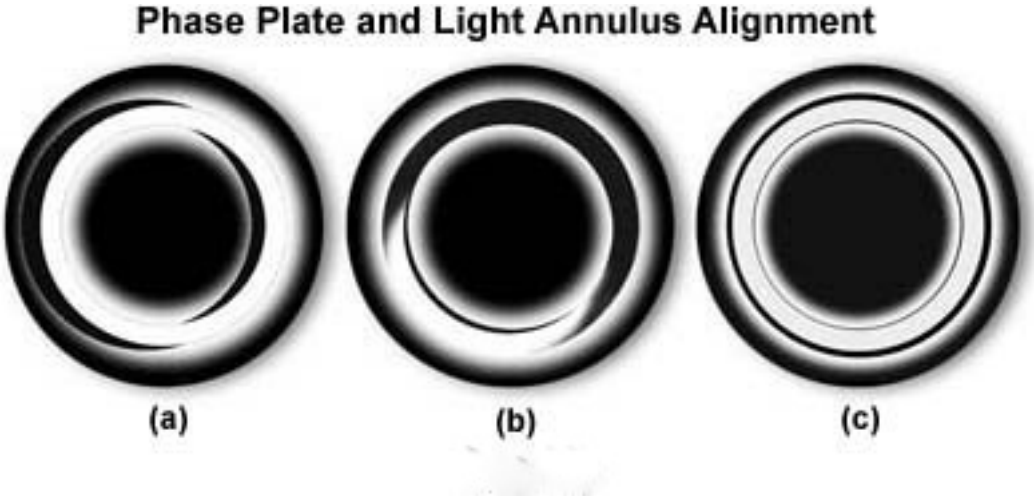
Centering Telescope

Figure 3

3025 SERIES PHASE CONTRAST SET-UP INSTRUCTIONS

1. Place the microscope in front of you and plug in your light source.
2. Mount the objectives clockwise (lowest magnification to highest magnification) onto the microscope nosepiece.
3. Install the turret phase condenser (Fig. 2) into the condenser holder located under the stage and tighten into place.
4. Position the turret phase condenser so that the "BF" on the rotatable annuli selector wheel is facing you. This places the condenser iris diaphragm in the light path. Rack up the condenser to the top and open the diaphragm.
5. Place a specimen slide on the stage and focus on it using the 10X objective.
6. Shift the slide to an area where the specimen is no longer visible; however, do not change the focus.
7. Rotate the phase turret wheel counter-clockwise until the "10" figure clicks into position. Note: There are two rotating wheels located at the front of the phase condenser. They are used to center the annuli in the condenser or phase rings.
8. Remove one of the eyepieces of the microscope and replace it with the phase telescope (Fig. 3). Note: The upper collar of the phase telescope can be moved in or out allowing you to bring the phase rings into focus.
9. Looking through the phase telescope you will see a dark field of view with two different rings (a & b), one bright (white) and the other black.
10. While viewing the rings through the phase telescope, adjust the two rotating wheels from the turret phase condenser until the narrower white ring is superimposed equidistant within the black ring (c).
11. Remove the phase telescope from the microscope and replace the eyepiece.
12. Bring the specimen back into the field of view and refocus the image until it is sharp. The image will be detailed and exhibit fine structural information.

NOTE: You must re-center the phase rings every time you change magnification by using a different objective. The turret wheel must be rotated so its number corresponds with the objective being used. To return to brightfield viewing, rotate the turret wheel until it is at the "BF" position.



TROUBLESHOOTING GUIDE

PHASE CONTRAST MICROSCOPY

PROBLEM	CAUSE	CORRECTIVE MEASURE
Poor phase contrast image is obtained	The condenser phase annular diaphragm image and the objective phase plate do not match.	Adjust the phase annular diaphragm so that it matches with the objective phase plate.
	The condenser phase annular diaphragm and the objective phase code do not match.	Insert the phase annular diaphragm with the same phase code as the objective into the optical path.
	The field diaphragm image is not focused on the specimen surface.	Move the condenser up or down to focus the image on the specimen surface.
	The phase difference of the specimen is too large.	Prepare the specimen using a different sealant and specimen thickness
	The type of phase objective does not match the phase difference of the specimen.	Select a phase objective suitable for the specimen.