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# 3012 Microscope
## Phase Contrast – Turret System

**SUPPLEMENTAL INSTRUCTIONS**

## 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.

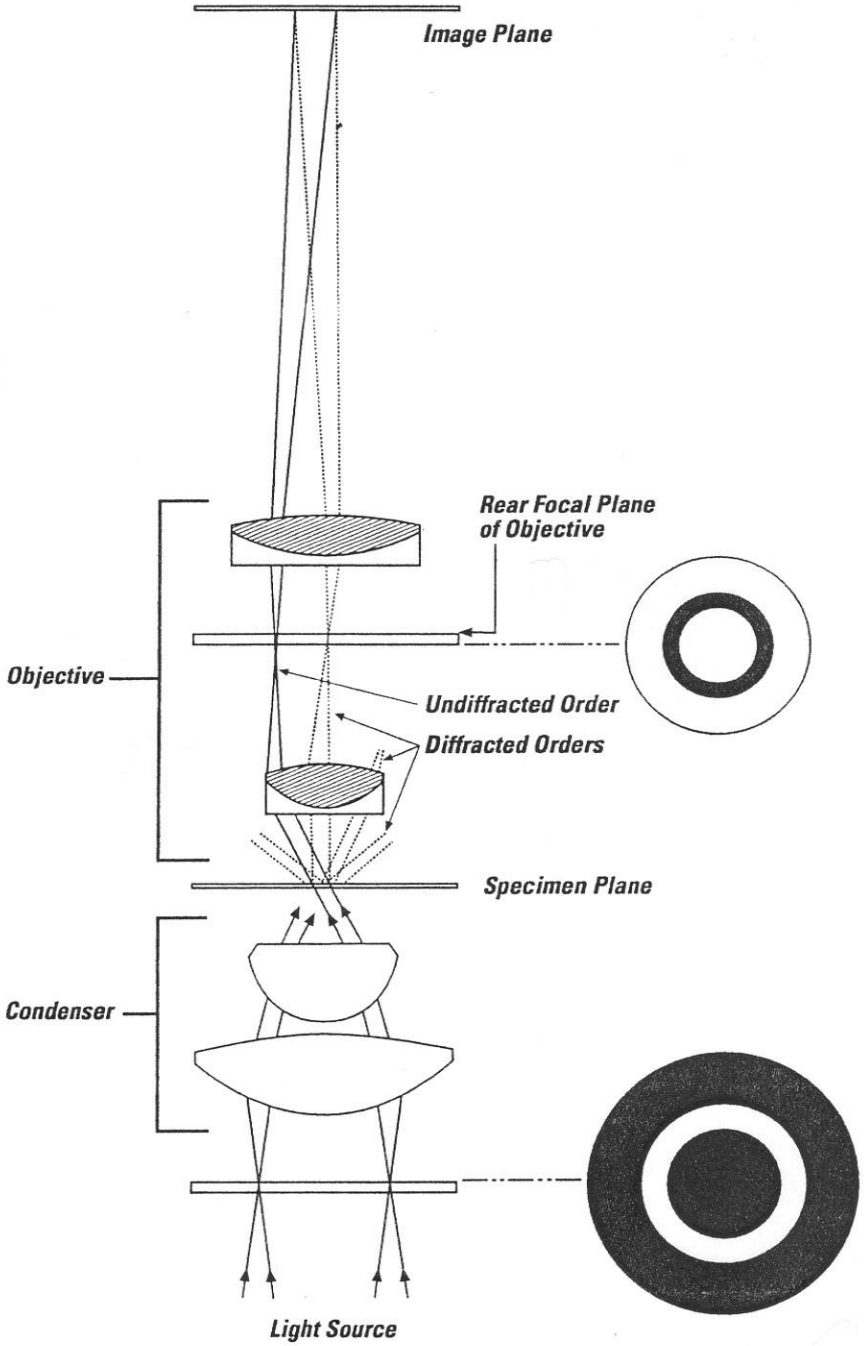
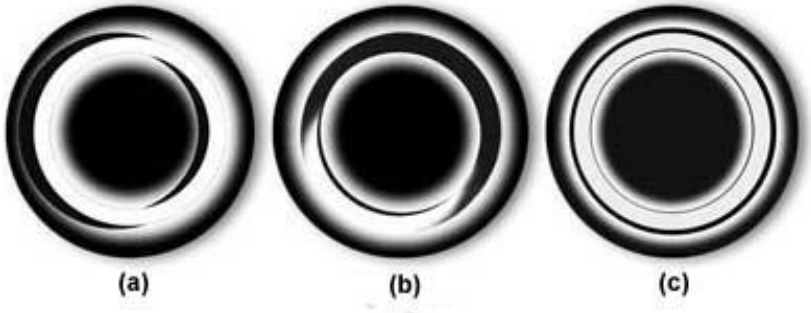


Image Plane
Rear Focal Plane of Objective
Objective
Undiffracted Order
Diffracted Orders
Specimen Plane
Condenser
Light Source

## Installation of the Phase Contrast Components & Alignment of the Condenser to the Optic Axis of the Microscope

1. Mount the Phase Contrast objectives onto the nosepiece— clockwise lowest to highest magnification.
2. Install the Phase Contrast Turret-style condenser onto the condenser carrier.
3. Raise the condenser carrier rack to its highest position.
4. Position the condenser turret rotating plate to the “BF” position. (Make certain the condenser diaphragm is full open).
5. Select the 10x Phase Contrast objective.
6. Place a “stained slide” specimen on the stage and focus the microscope.
7. Close the field diaphragm, then lower the condenser until an image of the field diaphragm comes into focus on the object plane.
8. Partially open the field diaphragm until the image of the diaphragm is close to the edge of the field of view. Now adjust the condenser carrier “centering screws” to align the condenser to the microscope optical axis.
9. Replace the “stained slide” with a phase contrast specimen (for example, a fresh “cheek cell” preparation).
10. Remove one eyepiece and install the supplied alignment telescope. Focus the telescope on the phase ring inside the 10x Phase Contrast objective.
11. Rotate the condenser-turret to its “10/20” position. As you observe through the telescope you will see two different rings, See illustration **Phase Plate and Light Annulus Alignment.**
12. The condenser-turret has two adjusting screws. These are used to align annular light rings in the condenser-turret to the corresponding phase ring in the objectives. Adjust the 10/20 annulus so that it is aligned – see Figure C below. (The 10/20 annulus will now function with ***both*** the 10x and optional 20x Phase objective).
13. Repeat the above annulus alignment procedure for the 40x and optional100x Phase objectives.

**Phase Plate and Light Annulus Alignment**



# TROUBLESHOOTING GUIDE

## PHASE CONTRAST MICROSCOPY

| PROBLEM | CAUSE | CORRECTIVE MEASURE |
|---|---|---|
| Poor phase contrast image is obtained | The condenser phase annulus image and the objective phase plate are not aligned | Adjust the phase annulus so that it is aligned with the objective phase plate. |
| | The condenser phase annulus and the objective phase code do not match. | Rotate the phase annulus selector wheel to the position that matches the objective in use |
| | The phase difference of the specimen is too large. | Prepare the specimen using a different refractive index immersion fluid |
| | The specimen cover glass is incorrect | Replace with 0.17mm thick cover glass |