

# **INSTRUCTIONS**

## **MOTIC Phase Contrast Equipment**

Since each objective is correlated with a certain setting of the annular stop turret, the illumination will always be exactly adjusted. The conditions are easy to control and reproducible at any time. The object is seen in Brightfield or Phase Contrast. Further, the full aperture of the condenser may be utilized in Brightfield. Therefore, it is possible to make full use of the resolving power of the objectives, without appreciable effect of the phase-ring during comparative examination.

### **Condenser**

The condenser has four built-in annular stops for Phase Contrast illumination and one blank setting for Brightfield. The annular stops can be centered individually by means of the centering keys. Two knurled centering screws on the condenser mount are used for centering the field diaphragm of which the condenser forms an image in the object plane (Köhler Illumination).

### **Phase Contrast Objectives**

Phase Contrast objectives differ from the conventional microscope objectives only in that they include a built-in annular phase plate. All objectives are supplied for positive phase contrast. They have the marking "Ph" engraved on their outer barrel.

### **Centering Telescope**

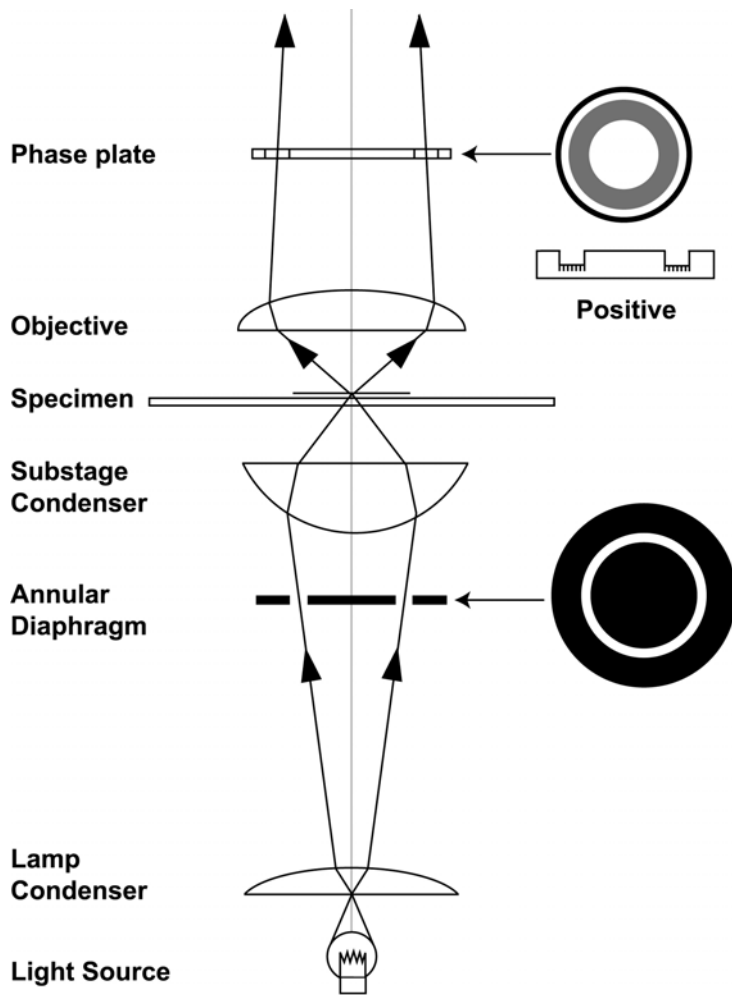
The centering telescope is interchangeable with the eyepiece, for the observation of the phase ring and the annular stop, and for the adjustment of the annular stop to the phase ring in the objective.

### **Filter**

The green filter is used for monochromatic observation.

### **Phase Contrast Microscopy**

Phase Contrast is produced by the combination of an annular stop located below the sub-stage condenser which directs a hollow cone of light through the specimen and a phase plate at the back focal plane of the objective. Some of the light passing through the transparent specimen is diffracted by slight differences in optical path and moves so as to be distributed over the whole aperture of the objective.



### **Schematic Diagram of Transmitted-Light Phase Contrast Microscope**

The balance of the light passes directly through the specimen as a cone of concentrated light towards coincidence with the ring of the phase plate. The phase plate alters the intensity and phase relationship of the diffracted and direct light so that when they recombine to form an image, invisible optical path differences are converted into visible light intensity differences.

With transparent specimens, positive phase contrast causes the area of greater optical density (thickness) to appear darker than their surroundings. Variations in optical thickness of about 3nm can be detected by using a green filter. This technique is useful for revealing subtle differences in between constituents within a specimen.

### **Microscopy**

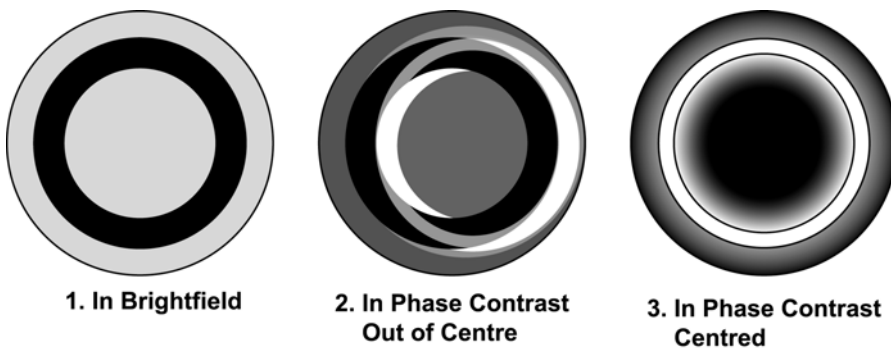
To center the Phase Turret Condenser:

- Insert the phase condenser into the sub-stage sleeve of the microscope. Fasten the condenser firmly with the clamp screw and raise it to its topmost position by means of the rack and pinion drive.
- Place the specimen on the stage.
- Revolve the condense turret to the position "0" and the objective Ph 1 (10/0.25) into the optical path.

- Focus on the specimen.
- Close the field diaphragm to approximately one-third of its aperture.
- Rotate the rack and pinion drive so that a sharp image of the field diaphragm is formed on the specimen surface.
- Bring the field diaphragm image to the center of the field of view by means of the condenser centering knurled screws.
- Open field diaphragm so that it is just visible at the margin of the field of view. If necessary, repeat centering.

To center the annulus of the Condenser with the Phase ring in the Objective:

- Set the condenser turret to the position Ph 1.
- Insert the centering telescope in one of the eyepiece tubes, and focus its eyelens on the dark phase ring by means of the knurled ring. The bright image of the condenser annular will also be visible. The bright ring of the condenser must now be coincident with the dark phase ring. Should the rings be out of center this must be remedied by means of the centering keys. Make the light ring exactly coincident with the phase ring by turning the centering keys.
- Check the centering with the three other objectives, by setting the annular stop turret at positions 2; 3; and 4 and turning in objectives 20/0.40; 40/0.65 and 100/1.25 Oil respectively.



**Phase ring and light ring as seen in the microscope**

- When immersion oil is used, it must be confined to the objective and cover glass, The condenser must not be immersed, i.e. no oil must be introduced between the condenser top lens and the underside of the microscope slide.
- Remove the centering telescope, insert the eyepiece.
- The microscope can now be used in the usual manner.

The essentials then, are the annular stop below the condenser and a phase plate located at the back focal plane of the objective. Centering and alignment are extremely important to produce the proper image.

### **Brightfield Microscopy**

Set the condenser turret to the position "0". Here the condenser functions as a Brightfield condenser, and the built-in iris diaphragm as an aperture diaphragm.



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