ACCU-SCOPE®

INSTRUCTIONS

3000-LED

SLIDER PHASE CONTRAST MICROSCOPY INSTRUCTIONS

PHASE ANNULI ARE PRE-CENTERED

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Slider Phase Contrast
Installation & Use Instructions

Phase contrast should be used for observing unstained specimens (i.e. cultured cells) at high contrast.

Prior to shipment your phase contrast set was assembled, tested and inspected at our distribution center in New York. The phase slider objectives and condenser were pre-centered and aligned by our technicians.

IMPORTANT: Before you can use the phase slider, ***you must remove the dust shield cover*** from the condenser housing and install the phase annuli slider in its place. (See **FIG. 1**)

Objectives

Green Filter

Phase Slider

IMPORTANT: The condenser has been intentionally installed in a slightly rotated orientation. This was done to prevent accidental contact on the stage control arm when using the annuli slider.

In the event the condenser is removed, it **MUST BE REINSTALLED** in the original orientation.

The **REFERENCE LINE** on the condenser must be ALIGNED WITH THE SET SCREW in the condenser holder.

REMOVING THE DUST SHIELD COVER

Slide the dust shield cover out from the condenser housing (**FIG. 1**). Be sure to keep it to reinstall it should you need to transport the microscope. **FIG. 2** shows the condenser housing with the dust shield cover removed.

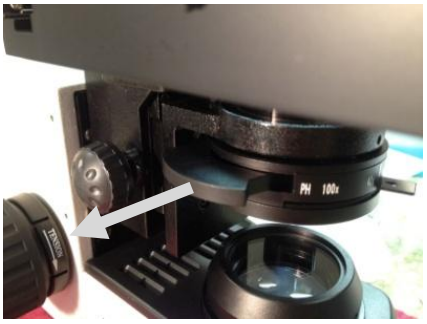


FIG. 1

INSTALLING THE PHASE SLIDER

Before inserting the Phase slider, it is recommended the iris diaphragm lever be set to PH on the condenser.

Insert the slider into the slot on the condenser as shown in **FIG. 3 & FIG 4**.

Move the slider to its position that corresponds to the phase objective in use (i.e., 40x objective = 40x position on slider).

Condenser Height Adjustment Knob

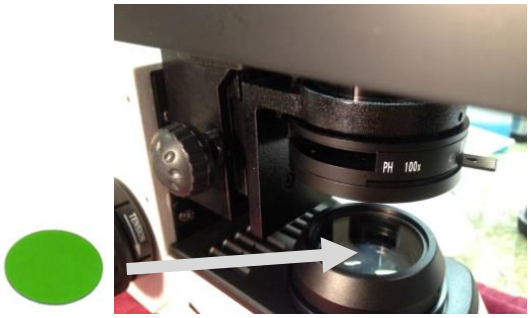
FIG. 2

IMPORTANT!
Raise the condenser to its uppermost position.

Refer to the instruction manual provided with the microscope on how to focus on a specimen.

GREEN FILTER

The included green filter can be mounted on top of the illuminator housing.



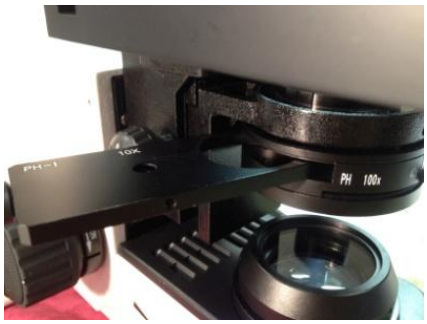


FIG. 3

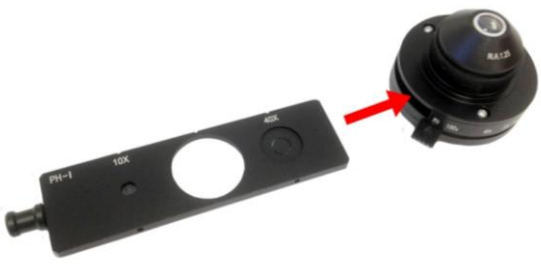


FIG. 4

PHASE CONTRAST MICROSCOPE

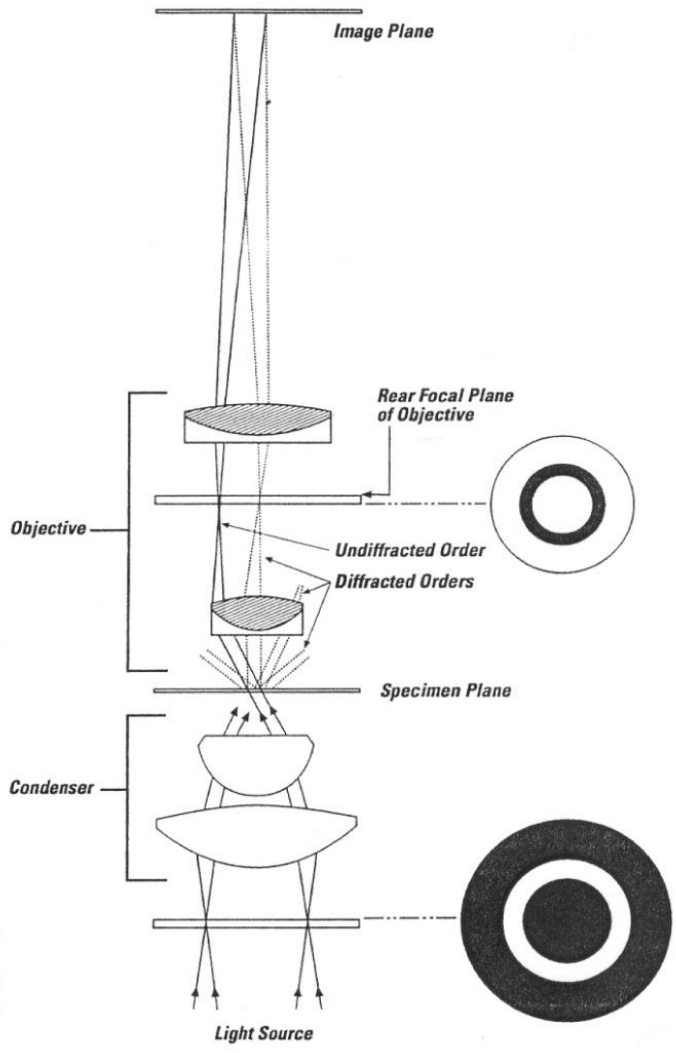
The normal microscopic object is seen because it has regions of varying density. In normal brightfield illumination a completely transparent specimen is very difficult to observe in detail because all areas of the specimen are equally dense. Darkfield illumination displays border effects in completely 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.



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