DIFFERENTIAL INTERFERENCE CONTRAST ATTACHMENT For TRANSMITTED LIGHT MODEL BH2-NIC

INSTRUCTION MANUAL

OLYMPUS

This instruction manual has been written for use of the differential interference contrast attachment Model BH2-NIC.
It is recommended to carefully read the manual for the microscope Model BHS or BHT as well as this manual so that you can fully understand and obtain the optimum integrated performance of this attachment with the microscope in use.

This attachment is specially designed for use with LB series optical elements including eyepieces, photo eyepieces, and objectives (no other than S Plan objectives, effective for the Nomarski method); otherwise no complete performance can be obtained from this attachment.

CONTENTS

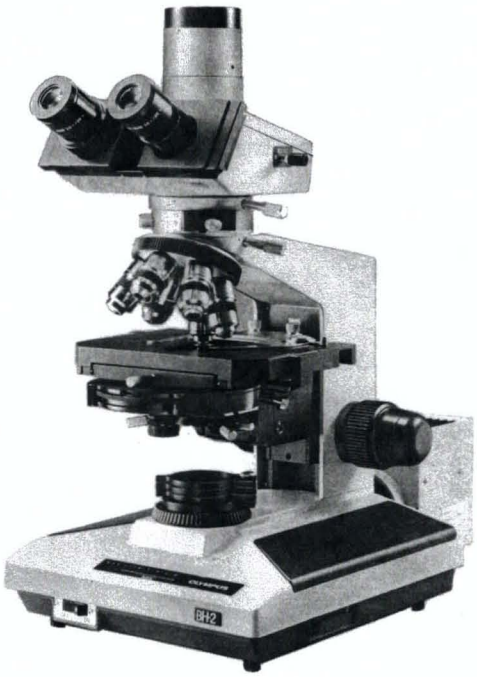
I. ABSTRACT

This differential interference contrast device incorporates a pair of birefringent crystal prisms as beam-splitters, with a shear between the two interfering beams smaller than the resolution limit of the objective used in the microscope, so that no double image is observed.

These attachments, used in conjunction with a BHS or BHT microscope, permit detection of minute difference of thickness and changes of internal refractive index, of unstained, transparent specimens.

By operating the birefringent crystal prism built in the intermediate tube, a most suitable contrast color can be chosen in accordance with purpose and specimen. The background color can be continuously changed in the order from dark to yellow, to purplish red, to blue or in the reversed order.

II. STANDARD COMPONENTS



BH2-NIC attachment mounted on Olympus microscope BHS

Components			BH2-NIC-1	BH2-NIC-2
Nomarski intermediate tube		BH2-NA	1	1
Nomarski condenser		BH2-NC	1	1
Phase contrast objectives	PC D Ach. 10X-PL PC D Ach. 40X-PL (spring-loaded)	(set of 2)	1	1
S Plan achromatic objectives	S Plan 10X S Plan 20X (spring-loaded) S Plan 40X (spring-loaded) S Plan 100X (spring-loaded) (oil)	(set of 4)	1	–
Centering telescope		CT-5	1	1
Filter		43IF550-W45	1	1
Auxiliary clamping wrench			1	1

III. PRINCIPLE OF NOMARSKI METHOD AND INTERFERENCE CONTRAST IMAGE

1. Principle

The differential interference contrast attachment, after Nomarski, is a two-beam shearing interference device developed on the basis of the Nomarski method, in which the amount of shear between two interfering beams is made less than the resolution limit of the objective used in the microscope, by means of birefringent crystals used as beam-splitters.

When a ray of light passes through a birefringent crystal, the optic axis of which is indicated by the double arrow, the ray is divided into two linearly polarized rays, an extraordinary ray which runs parallel to the plane of the diagram, and an ordinary ray, whose plane of vibration is perpendicular to that of the extraordinary ray, as shown in Fig. 1.

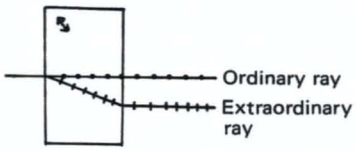


Fig. 1

In Fig. 2, a modified Wollaston prism consists of two wedge-like uniaxial crystals cemented together, whose optic axes are at a right angle to each other. If a ray of linearly polarized light enters the prism, it will be divided into two rays traveling in different directions.

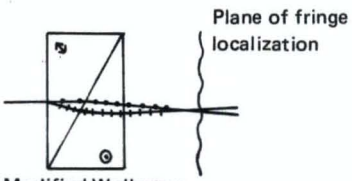


Fig. 2

⇅ & ◉ indicate the directions of optic axes; for the direction parallel with the plane of the diagram, and ◉ for the direction perpendicular.

After emerging from the prism, the two rays intersect at a fixed point. The plane, which includes the point of this intersection, is called the plane of "fringe localization".

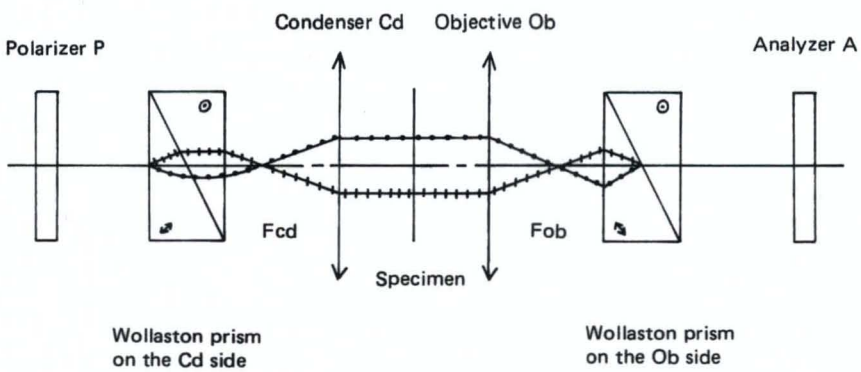


Fig. 3

Fig. 3 is a diagram of a light path in which a ray of light emitted from the light source is linearly polarized after it passes through the polarizer P. Entering the Wollaston prism on the condenser side, it is divided into two rays of linearly polarized light. In order to equalize the intensities of the rays and therefore to maximize the contrast of the interference fringes, the Wollaston prism must be placed with its optic axis at 45° to the direction of vibration of the linearly polarized light incident on the prism. The two divided rays intersect at a point on the plane of fringe localization, which is calculated to coincide with the focal plane Fcd of the condenser Cd. They then pass through the condenser, become parallel to each other with a slight lateral separation and illuminate the specimen. The two rays are transmitted through the specimen and the objective which recombines the separate rays at the plane of fringe localization of the second Wollaston prism on the objective side, at the rear focal plane Fob of the objective Ob. The combined rays do not interfere because their directions of vibration are perpendicular to each other. In order to observe interference fringes, an analyzer must be introduced behind the Wollaston prism on the objective side.

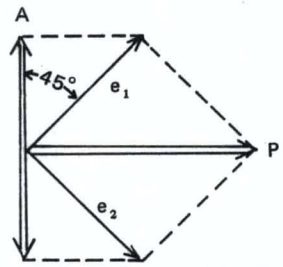


Fig. 4

Fig. 4 indicates that the analyzer is placed in a "crossed-filter" position with the polarizer. After the linearly polarized waves "e_1" and "e_2" as divided by the Wollaston prism on the condenser side pass through the different paths in the specimen as shown in the vector diagram, their components along their directions of polarization will form interference fringes having maximum visibility, according to the shear between the two interfering components. If the polarizer P is rotated 45° from the "crossed-filter" position, either "e_1" or "e_2" will be blocked, therefore, interference fringes cannot be formed and an image similar to a bright field image is observed.
If the plane of fringe localization coincides with the rear focal plane of the objective, the spacing of the fringes becomes infinitely large so that one color fringe fills the entire field of view. When this condition is fulfilled, interference contrast is obtained.

2. Interference Contrast Image

From the view of geometrical optics, two sheared images should be formed in the image plane of the objective, in other words, in the focal plane of the eyepiece. However, they are not recognized as a double image because the amount of the shear is too small to be resolved.

Now consider a specimen with refractive index N_2 at position A, smaller than the refractive index N_1 at the surroundings B, as shown in Fig. 5. After passing through the specimen, the wavefront W of the light is divided into two wavefronts, one at position A and the other at position B, advancing at A by $d\,(N_2 - N_1)$ with a sheared amount Δ.

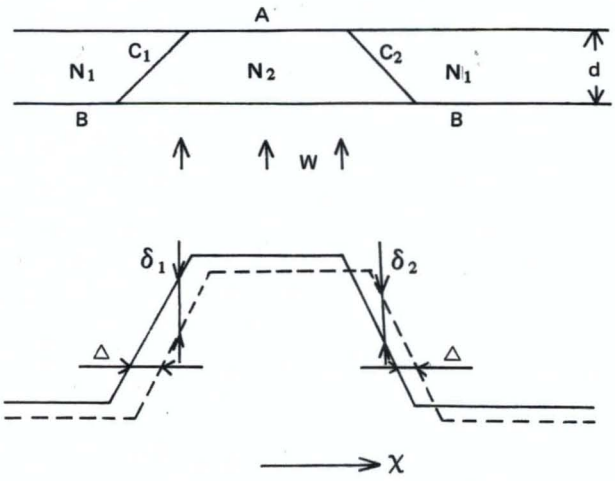


Fig. 5

There occurs a difference between the optical path lengths (or products of the refractive index "**N**" and thickness "d") at C_1 and C_2. This difference depends on phase differences δ_1 and δ_2 at C_1 and C_2, respectively.
There is no difference in background colors at A and B, while at C_1 and C_2, the background colors differ, depending upon phase differences at δ_1 and δ_2.

$$\delta_1 = \Delta\left(\frac{\partial w}{\partial X}\right)_1 \qquad \delta_2 = \Delta\left(\frac{\partial w}{\partial X}\right)_2$$

$\frac{\partial w}{\partial X}$ is the differential coefficient of the wavefront. These equations imply that the path difference is equal to the product of the shear Δ and the differential coefficient, from which is derived the name of "differential interference". By moving the prism in the direction as indicated by the arrow in Fig. 6, the path difference between two wavefronts is changed; accordingly the color of the background changes.

Fig. 7 shows the relation between the wavefronts when path difference δ_0 is introduced by the movement of the prism. In this case, path differences at C_1 and C_2 are given by

$$\delta'_1 = \delta_0 + \delta_1 \qquad \delta'_2 = \delta_0 + \delta_2$$

By adjusting the path difference δ_0, it is possible to observe images similar to dark field, relief-like images or sensitive color images. Thus, the best result will be obtained by selecting the type of the image suitable for the specimen to be observed.

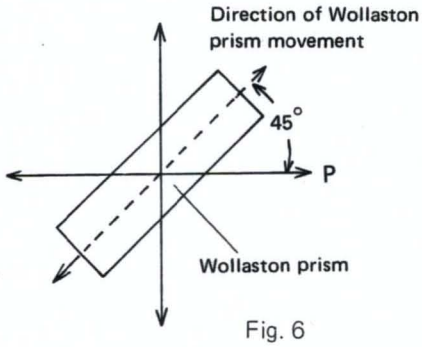


Fig. 6

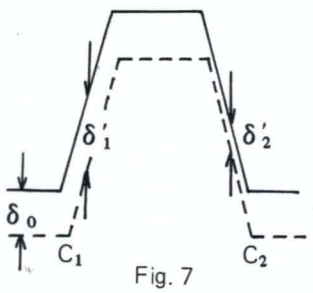


Fig. 7

3. Characteristics and Applications of Nomarski and Phase Contrast Methods

1) Characteristics

① Generally the larger the phase difference, the more conspicuous the halos, which is a drawback of the ordinary phase contrast method, making distinct observation of contours more difficult. The Nomarski method does not cause halos, so that a clearer definition of image details is obtained.

② The Nomarski method permits utilization of the full numerical aperture of the objective, which results in exceptional image brightness, and resolution almost double that of the light annulus used in phase contrast microscopy.

③ The depth of focus is smaller in differential interference contrast than in phase contrast. This prevents disturbing out-of-focus effects, as with phase contrast, and permits layer by layer scanning of specimens, commonly called "optical sectioning".

④ Interference colors, or grey shadings, can be seen in proportion with the gradient of path differences of the specimen. Phase contrast helps to determine a high or low refractive index of a specimen detail by the appearance of halos, according to the positive or negative contrast of the objective in use.

⑤ As the shearing direction is restricted in accordance with the sliding direction of the Wollaston prism, it is preferable to use a rotatable stage to orient the specimen in the direction best suited for optimum resolution.

⑥ For the observation of anisotropic objects, it is recommended to apply phase contrast rather than interference contrast for better results.

2) Applications

The interference contrast method renders a sharply defined, relief-like, image with excellent contrast in a wide range of interference colors. This method permits observation of unstained transparent objects like phase contrast, which makes it useful for observations in histology, cytology, biology, anatomy, etc.

IV. SPECIFICATIONS

■ **Nomarski Intermediate Tube**

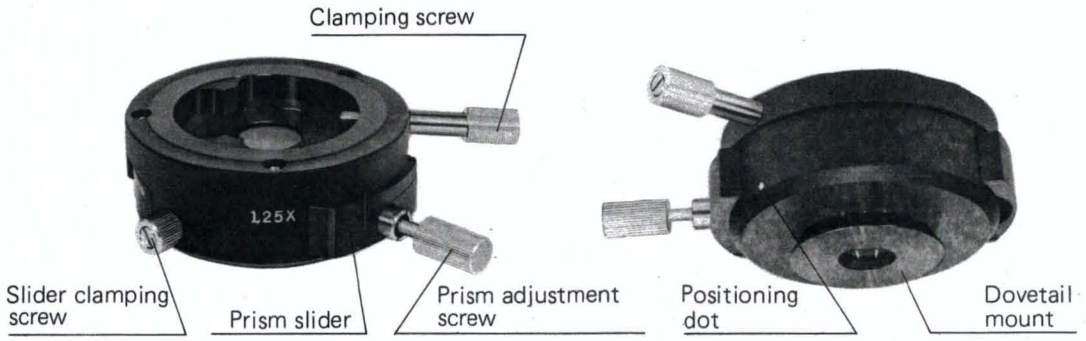
Tube magnification 1.25X; incorporates the modified Wollaston prism and analyzer with depolarizer, both removable.

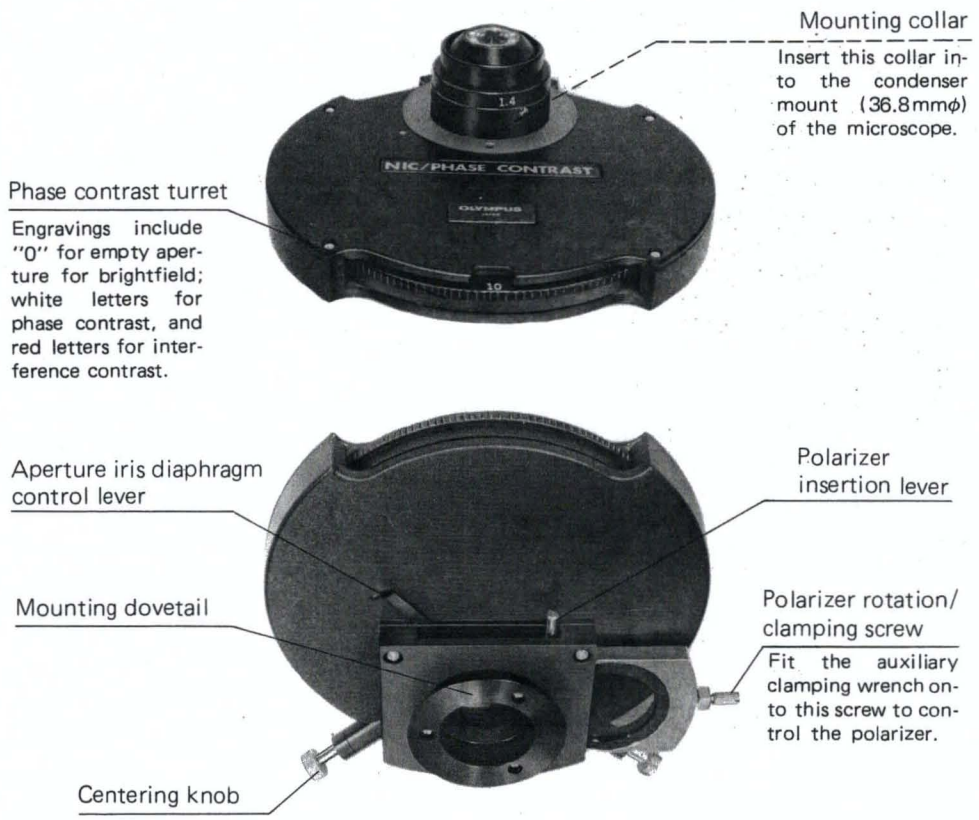
■ **Nomarski Condenser**

Achromatic/aplanatic condenser N.A. 1.40.
Phase contrast and interference contrast turret with 7 apertures, including one empty opening for brightfield; 4 modified Wollaston prisms (for 10X, 20X, 40X and 100X objectives) and 2 light annuli (for 10X and 40X objectives); centerable by centering screws.
Polarizer built-in, rotatable and removable.
Iris diaphragm built-in, with aperture adjustment lever.

V. DESCRIPTION OF VARIOUS COMPONENTS

A. Nomarski Intermediate Tube BH2-NA





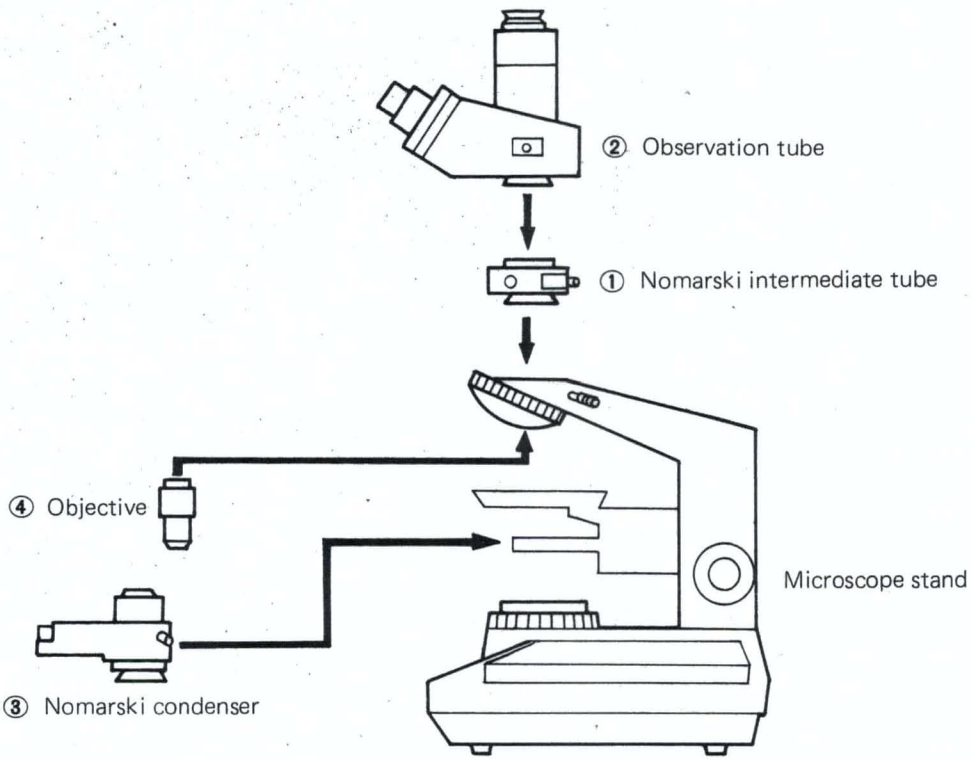
C. Centering Telescope CT-5

D. Auxiliary Clamping Wrench

- Fit this wrench onto the polarizer rotation screw to rotate or lock the polarizer in position.
- When the Nomarski condenser is inserted into the condenser mount of the microscope, clamp the screw with this wrench. After tightening, remove the wrench.

VI. ASSEMBLY

Prior to assembly of this attachment, read the instruction manual for the BHS/BHT microscope.



VII. OPERATION

1. Condenser Centration

1) Loosening the clamping screw of the interference contrast prism slider, pull out the slider.
2) Disengage the polarizer from the light path by means of the polarizer insertion lever.
3) Rotate the turret, until the engraving "0" can be seen through the central window in the condenser front, and open the aperture iris diaphragm fully.
4) Place a specimen on the stage, bring the 10X objective into the light path, and bring the specimen in focus.
5) Stop down the field iris diaphragm with the knurled ring on the microscope all the way.
6) While looking through the eyepieces, move the condenser up and down with the condenser height adjustment knob to focus on the image of the field iris diaphragm.
7) While widening the diameter of the field iris diaphragm progressively, manipulate the condenser centering knobs to bring the diaphragm image into the center of the field of view. (Fig. 8)

   When the polygonal image of the iris diaphragm becomes inscribed in the field, slightly increase the diameter of the field iris diaphragm until it is just outside the field of view.

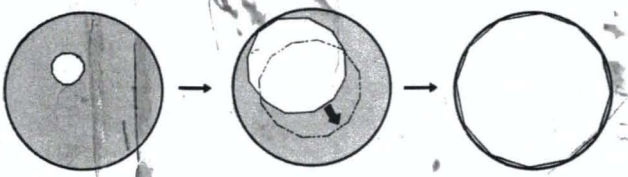
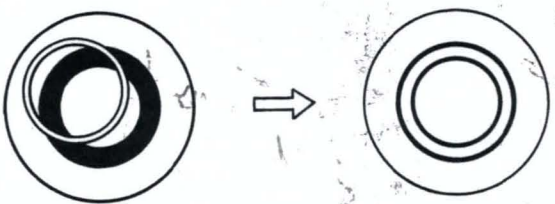


Fig. 8

2. Aligning Phase Annulus and Light Annulus

1) Mount the phase contrast objective 10X on the nosepiece and swing it into the light path.
2) Rotate the phase contrast turret until the engraving "10" (white) can be seen through the central window.
3) Place a specimen on the stage and bring it into approximate focus.
4) Remove the eyepiece, and insert the centering telescope CT into the eyepiece tube.
5) Looking through the centering telescope, rotate the top lens assembly of the CT until the bright ring (light annulus) and the dark ring (phase annulus) are sharply focused.
6) While pressing each centering knob ① at the bottom of the condenser with your fingers, rotate the centering knobs until bright ring and dark ring are concentric and superimposed in the field of view through the CT. (Fig 9)



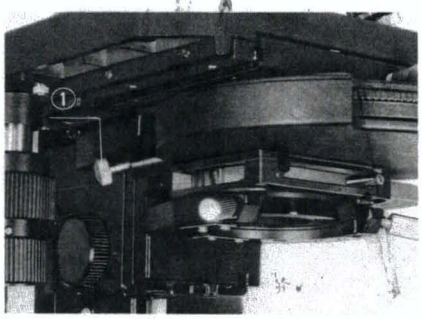


Fig. 9

For centration of the 40X objective, engage the phase contrast objective 40X, and rotate the turret until the engraving "40" can be seen through the central window, then commence centration as mentioned above.

7) Remove the centering telescope and insert the eyepiece back into the eyepiece tube.

3. Polarizer Centration

1) Push in the prism slider in all the way, and clamp.
2) Rotate the turret until the engraving "0" appears in the central window.
3) Screw in the prism adjustment screw all the way.
4) Move the polarizer insertion lever ① to the left all the way to engage the polarizer. (Fig. 10)
5) Swing the 10X objective into light path, and replace the eyepiece with the CT.
6) Fit the auxiliary wrench onto the polarizer rotation screw ② and looking through the CT, adjust the wrench position so that a black interference fringe can be seen. When this fringe is most sharply seen, the polarizer is in the correct position. (Fig. 11)
7) Then, tighten the polarizer rotation screw.

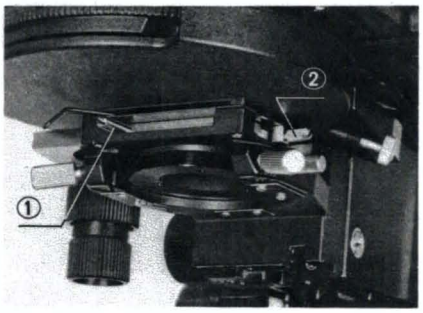


Fig. 10

Fig. 11

4. Light Path Selection

Mode of observation	Objective	Turret setting of condenser	Polarizer	Prism slider
Brightfield	10X ~ 100X	0	OUT	OUT
Interference contrast	S Plan 10X	10 (red)	IN	IN
	S Plan 20X	20 (red)		
	S Plan 40X	40 (red)		
	S Plan 100X	100 (red)		
Phase contrast	PC D Ach. 10X-PL	10 (white)	OUT	OUT
	PC D Ach. 40X-PL	40 (white)		

Note: Any objective other than those mentioned above cannot be used for interference contrast or phase contrast observation.

5. Interference Contrast

After completing the polarizer adjustment so that the black interference fringe is most sharply seen, take the following steps:

1) Choose a most suitable contrast color in a range from 0 order (black) to second order (blue) (or 0 to 700nm) by rotating the prism adjustment screw.

- When the background is dark, darkfield-like observation is possible.
- When the background is made gray, the visual sensitivity to detect small path difference as a relief-like image becomes most pronounced.
- When the background color is magenta, a change of path difference can be most sensitively detected as a change of color.

★ **Bear in mind that even the smallest amount of contamination on the surface may be shown up because of the extreme sensitivity in differential color contrast.**

2) Since optimum resolution is achieved only if the direction of the details to be observed is parallel to the prism shear, it is recommended to use a rotatable stage (Model BH2-SRG).

6. Phase Contrast

Remove the prism slider and the polarizer from the light path.

1) Mount the phase contrast objective on the nosepiece.
2) Center the light annulus. (Refer to "2. Aligning Phase Annulus and Light Annulus" in page 8.)
3) After inserting the eyepiece into the eyepiece tube, you can perform phase contrast microscopy.
4) The green filter provided can be mounted on the filter mount of the microscope base. It increases contrast in your observation or monochromatic photomicrography.

7. Brightfield

Remove the prism slider and the polarizer, and rotate the phase contrast turret until the engraving "0" is seen through the central window, before you start your brightfield observation.

8. Magnification at Observation and Photomicrography

1) Observation magnification = Obj. power X Eyepiece power X 1.25
2) Photomicrography magnification = (for 35 mm camera)
   Obj. power X NFK photo eyepiece power X 1.25
   (for large format camera)
   Obj. power X NFK photo eyepiece power X 3 X 1.25

VIII. TROUBLESHOOTING

If you are unable to obtain full performance from your instrument, please consult with the table below as pointers for troubleshooting.

Troubles	Causes	Remedies
No interference color can be seen.	The polarizer is not in the light path.	Insert the polarizer into the light path.
	The phase contrast turret is not correctly aligned.	Align the turret correctly.
	The prism slider is not in the light path.	Insert the slider into the light path.
The interference color can be seen irregularly.	The height of condenser is not correctly adjusted.	Bring the condenser into focusing position accurately.
	The phase contrast turret is not correctly positioned to the magnification of objective in use.	Match the magnification of objective and turret.
	No designated objective is used.	Use the designated objectives.
	The Nomarski intermediate tube is mounted in the wrong direction.	Correct the direction of the tube.
	Objectives of a wrong type are used.	Use S Plan objectives.
No image of field diaphragm is seen.	The slide glass is too thick.	Use a slide glass less than 1.2 mm thick.
The light annulus and phase annulus are not matched.	No designated phase objective is used.	Use designated phase objectives.

MEMO

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