# INSTRUCTIONS

# IMT2-NIC2

**DIFFERENTIAL INTERFERENCE CONTRAST ATTACHMENT FOR TRANSMITTED LIGHT AFTER NOMARSKI**

**WARNING**

*This instruction manual describes the operation of the differential interference contrast attachment after Nomarski Model IMT2-NIC2. It is recommended that you read the instruction manual for the microscope IMT-2 to be used with this attachment as well as this manual, so that you can fully understand and obtain their optimum integral performance.*

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# BEFORE USE

Observe the following procedures carefully:

## 1. Operation

① The microscope is a precision instrument. Always handle it with care, and avoid rough handling and impacts.

② Avoid exposure to direct sunlight, high temperature and humidity, dust and vibration.

③ Use objectives as designated in the specifications on page 1. Use of optical components other than designated will result in unsatisfactory performance of the microscope.

④ Use glass vessels of a flat bottom, since plastic vessels are subject to optical strains and ineffective to obtain an interference image of proper contrast.

⑤ Water drops on a vessel cap block effective differential interference or phase contrast observation. If an open vessel is used, cap it at the time of observation. In case of a sealed vessel, moisten inside the vessel cap with culture solution to remove dewdrops.

## 2. Maintenance and Storage

① Lens surfaces must always be kept clean. Fine dust on lens surfaces should be blown off by means of a hand blower. Carefully wipe off oil or fingerprints on the lens surfaces with gauze moistened with a small amount of xylene or a cleaning medium (alcohol and ether 3 : 7).

② Do not use organic solution to wipe the surfaces of various components.
Plastic parts, especially, should be cleaned with a neutral detergent.

③ Be careful not to spill the culture solution, etc. If spilt, it should be wiped off immediately. It is recommended to use a waterproof cover optionally available.

④ When objectives are not screwed in the nosepiece apertures, close the empty apertures with dust plugs, which will protect the lenses located in the lower light path from dust, culture solution, etc.

⑤ When not in use, the microscope should be covered with the dust cover provided.

# CONTENTS

# 1 ABSTRACT

This differential interference contrast (DIC) attachment, used in conjunction with the inverted tissue culture microscope IMT-2, permits not only differential interference contrast, but also phase contrast, simple polarization and brightfield observation, merely by turret control operation without any condenser change. Also IMT-2 permits fluorescence observation at the same time.

# 2 STANDARD CONFIGURATIONS

| Component | | IMT2-NIC2-1 | IMT2-NIC2-3 |
|---|---|---|---|
| DIC attachment IMT2-NA2 and long working distance DIC turret condenser IMT2-LWDNC | IMT2-NAC2 | O | O |
| S Plan achromatic objective 10X | SPL10X | O | O |
| Long working distance D Plan achromatic objective 20X (correction collar) | LWDCDPL20X | O | O |
| Long working distance D Plan achromatic objective 40X (correction collar) | LWDCDPL40X | O | O |
| S Plan achromatic phase contrast objective 10X | PCSPL10XPL | O | |
| Long working distance D Plan achromatic phase contrast objective 40X (correction collar) | LWDCDPL40XPL | O | |

# 3 SPECIFICATIONS

| Item | | Description |
|---|---|---|
| Type | | Differential interference contrast attachment for transmitted light after Nomarski. |
| Contrast method | | Polarized two-beam shearing interference contrast developed on the basis of the Nomarski method. |
| DIC turret condenser IMT2-LWDNC | Condenser | Long working distance DIC turret achromatic condenser:<br>N.A. 0.55, W.D. 21 mm, Focal distance 24 mm |
| | Turret | Phase contrast and interference contrast turret with 6 apertures, including one empty opening for brightfield; 3 modified Wollaston prisms (for 10X, 20X, 40X objectives) and 2 light annuli (for 10X and 40X objectives) |
| | Light annuli | Centerable by centering screws for each objective. |
| | Polarizer | Built-in the slider, with fine adjustment of vibration direction. |
| | Aperture iris diaphragm | Built-in iris diaphragm, adjustable from 3.5mm to 28mm dia. |
| DIC attachment IMT2-NA2 | Magnification factor | 1X. |
| | Nomarski prism | Sliding direction at right angles with optical axis. |
| | Interference colors | Adjustable with fine adjustment knob (from gray to purplish red, or in reversed order). |
| | Analyzer | Provided with depolarizer; and removable.<br>Permits simple polarizing observation in conjunction with polarizer built-in the Nomarski condenser. |
| Objectives | Differential interference contrast | SPL10X: N.A. 0.30, W.D. 7.5 mm<br>LWDCDPL20X: N.A. 0.40, W.D. 3.0 mm<br>(with correction collar)<br>LWDCDPL40X: N.A. 0.55, W.D. 1.9 mm<br>(with correction collar) |
| | Phase contrast | PCSPL10XPL: N.A. 0.30, W.D. 7.5 mm<br>LWDCDPL40XPL: N.A. 0.55, W.D. 1.9 mm<br>(with correction collar) |

# 4 CHARACTERISTICS AND APPLICATIONS OF DIFFERENTIAL INTERFERENCE CONTRAST AND PHASE CONTRAST

## 1. Characteristics (Comparisons between these two contrast methods)

① The ordinary phase contrast method causes halos; generally the larger the phase contrast, the more conspicuous the halos, which is a drawback of the ordinary phase 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. To give an example:

| Specimen form | Pertinent method |
|---|---|
| Spherical (e.g. ovum) | Differential interference contrast |
| Flat | Phase contrast |

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.

④ Interference colors or gray 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.

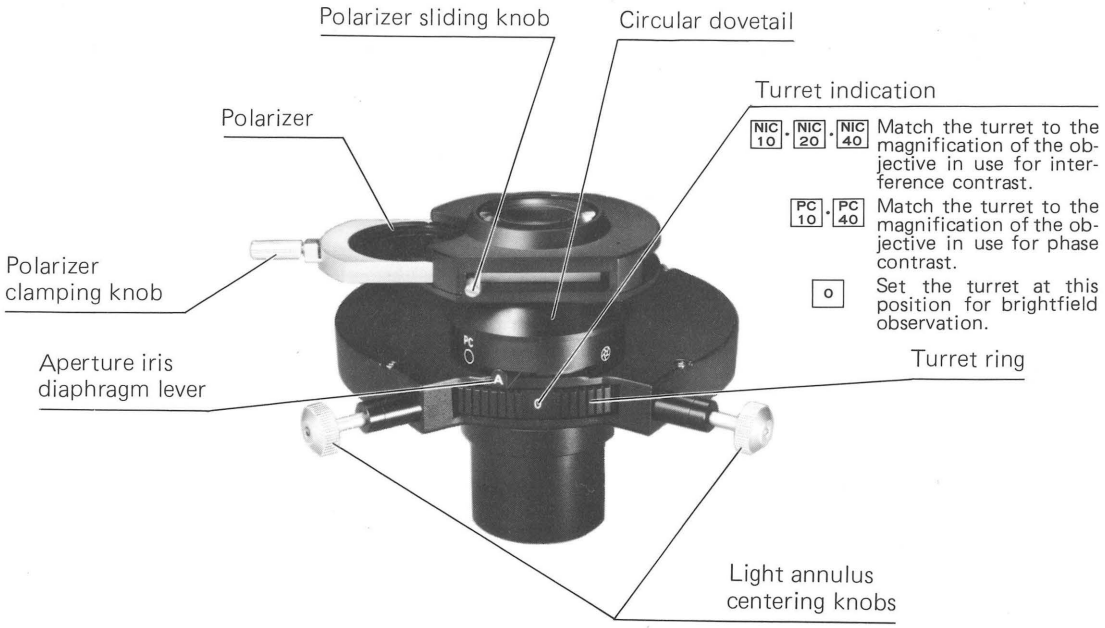
⑥ For use of a plastic vessel, it is recommended to apply phase contrast rather than interference contrast for better results.

## 2. Applications

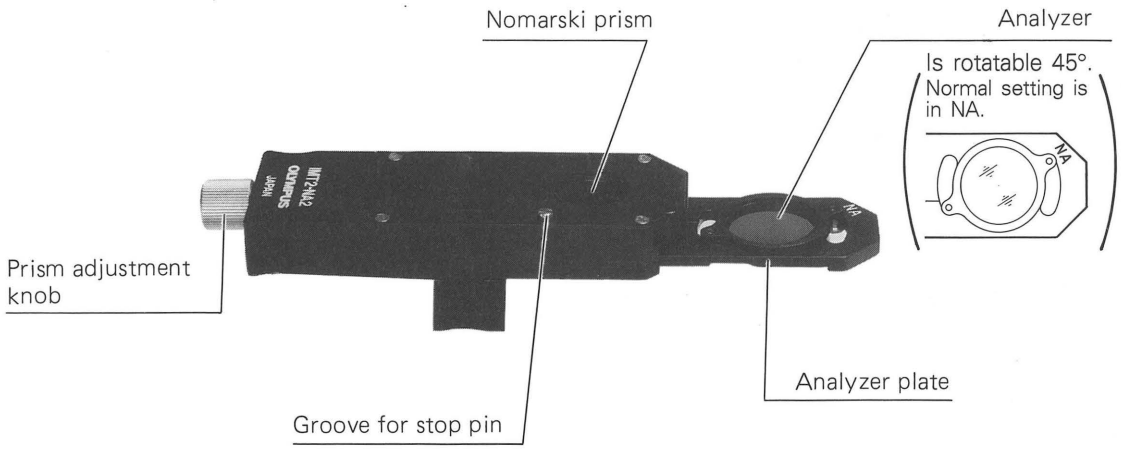
The interference contrast method renders a sharply defined, relief-like image with excellent contrast in a wide range of interference colors. This method permits observation of unstained transparent objects like phase contrast, which makes it useful for observations in histology, cytology, biology, anatomy,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

# 5 NOMENCLATURE

## A. DIC Turret Condenser IMT2-LWDNC




## B. DIC Attachment IMT2-NA2



# 6 ASSEMBLY

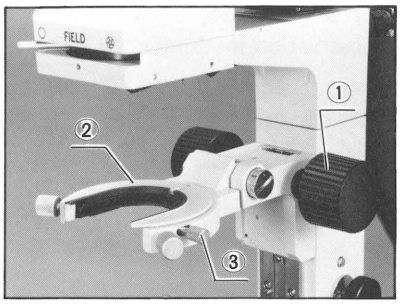



Fig. 1

### ❶ Mounting the DIC Turret Condenser (IMT2-LWDNC) (Fig. 1)

1) Tighten the condenser holder clamping knob ① .

2) Insert the circular dovetail of the condenser into the condenser holder ② and clamp the condenser with clamping knob ③ .

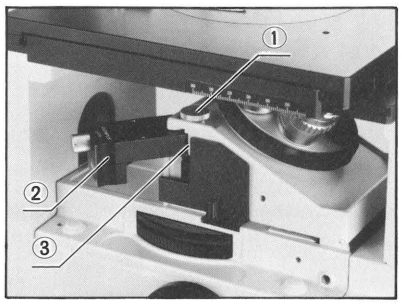



Fig. 2

### ❷ Mounting the DIC Attachment (IMT2-NA2) (Fig. 2)

1) Loosen the DIC attachment clamping knob ① , and remove the dust plug.

2) Insert the DIC attachment ② into the Nomarski slider insertion slot ③ .

3) Lightly tighten the attachment clamping knob.

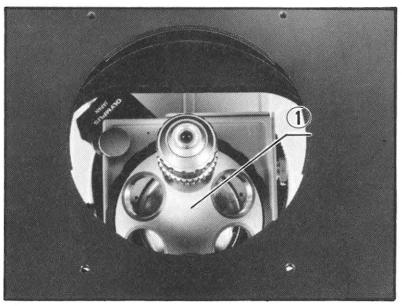



Fig. 3

### ❸ Mounting the Objectives

1) Remove the stage insert plate from the stage, and screw the objectives into the nosepiece ① . (Fig. 3)

★ **It is convenient to mount the objectives in the order of magnifications corresponding to the magnifications of Nomarski prisms and light annuli as indicated on the turret of the DIC turret condenser.** (Fig. 4)

An example of mounting order of the objectives:

| Order | Objectives | |
|---|---|---|
| ① | LWD CD Plan | 40X |
| ② | LWD CD Plan | 20X |
| ③ | S Plan | 10X |
| ④ | LWD CD Plan | 40X-PL |
| ⑤ | S Plan | 10X-PL |
| ⑥ | Dust plug | |

★ **Close each empty aperture in the nosepiece with a dust plug provided.**

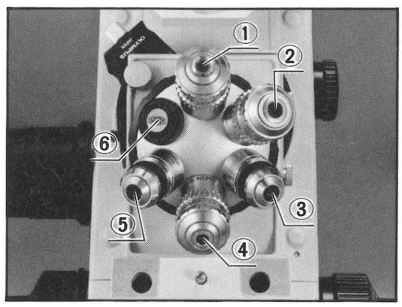



Fig. 4

# 7 PREPARATION

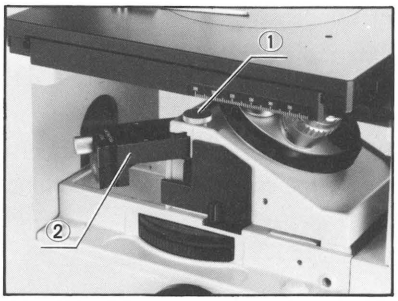



Fig. 5

**1. Centration of the DIC Turret Condenser (Basic procedure for all observation methods)**

1) Loosen the DIC attachment clamping knob ① slightly and pull out the DIC attachment ②, but do not let it fall out. (Fig. 5)

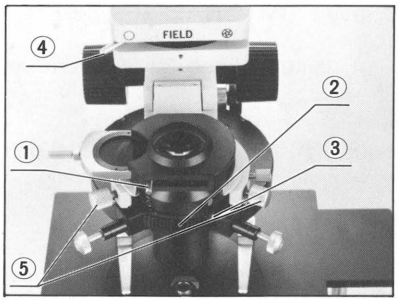



Fig. 6

2) Slide the polarizer sliding knob ① to the left side, and remove the polarizer out of the light path. (Fig. 6)

3) Align the turret ② to the brightfield position "0".

4) Place a specimen on the stage and bring it into focus with the 10X objective.

★ **It is suggested to stop down the aperture iris diaphragm ③ for easier focusing of unstained specimens.** (Fig. 6)

5) Stop down the field iris diaphragm to the minimum by means of the lever ④. Rotate the condenser height adjustment knob until the image of the field diaphragm is visible sharply in the field of view. (Figs. 6, 7)

6) Bring the image of the field diaphragm into the center of the field by means of the condenser centering knobs ⑤. Re-open the diaphragm until the small ring of the diaphragm becomes a larger polygonal ring around the circular edge of the field. (Figs. 6, 7)

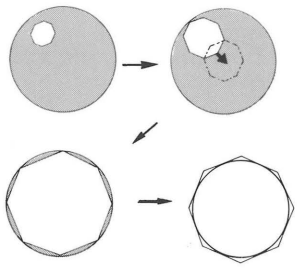


Fig. 7

**2. Light Annulus Centration (Phase contrast observation)**

The IMT-2 microscope adopted the individual centration system of each phase annulus, so that strict centration of the light annulus can be achieved with each objective. Therefore, match the light annulus to the phase objective magnification whenever objectives are changed, and re-centration is not necessary once the initial centration has been accomplished.

★ **Re-centration, however, is required when the bottom of a culture vessel is not flat and even.**

1) Swing in the desired objective and focus on the specimen.

2) Rotate the magnification changer ① to the CT position. (Fig. 8)

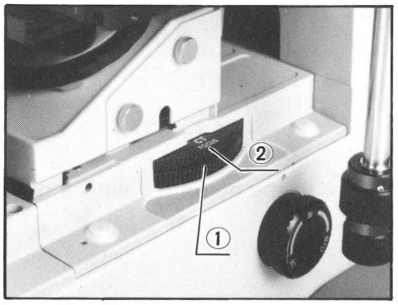



Fig. 8

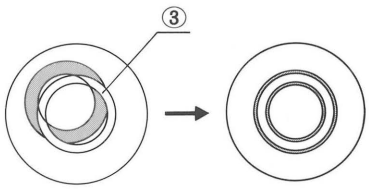



Fig. 9

3) Focus on the phase annulus ③ with the focus ring ②. (Figs. 8, 9)

4) Rotate the condenser turret until the magnification of the objective in use appears in the front of the observer.

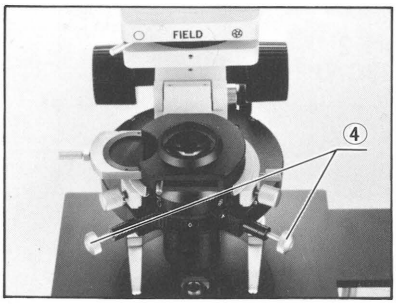



Fig. 10

5) Press the light annulus centering knobs ④ and rotate them until the light and phase annuli are concentric and superimposed, then slowly disengage the centering knobs ④. (Fig. 10)

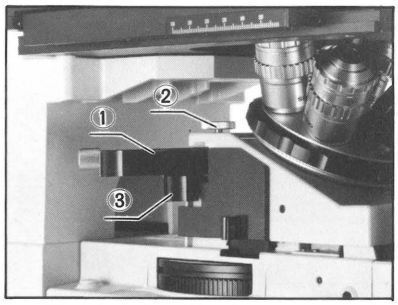



Fig. 11

**3 Adjustment of the Polarizer**
**(Simple polarization observation)**

1) Pull out the DIC attachment ① and tighten the clamping knob ②. (Fig. 11)

2) Push in the analyzer plate ③ all the way. (Fig. 11)

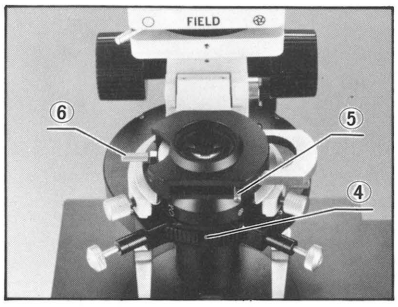



Fig. 12

3) Rotate the condenser turret ④ to position "0". (Fig. 12)

4) Slide the polarizer sliding knob ⑤ to the right completely to engage the polarizer. (Fig. 12)

5) Looking through the eyepiece, slightly move the polarizer clamping knob ⑥ horizontally back and forth to change the brightness of the field of view. When the optimum extinction is obtained, tighten the clamping knob ⑥. (Fig. 12) At this position, the vibrating directions of polarizer and analyzer are crossed at right angles, where an interference color can be seen if the specimen is birefringence.

# 8 OPERATION

## 8–1 Summary of Putting the Microscope in Operation

The microscope operation varies according to the observation methods, including differential interference contrast, phase contrast, simple polarization and brightfield. Refer to the table below for each observation.

**Operation Table**

| Relevant component | | Observation | | | |
|---|---|---|---|---|---|
| | | Phase contrast | Brightfield | Interference contrast | Simple polarization |
| Objective | | S Plan 10X-PL<br>LWDCD Plan 40X-PL | S Plan 10X<br>LWDCD Plan 20X<br>LWDCD Plan 40X | | |
| DIC turret condenser | Turret | 10 PC<br>40 PC<br>(Center the light annulus.) | 0 | 10 NIC<br>20 NIC<br>40 NIC | 0 |
| | Polarizer | OUT | | IN<br>(Attain the crossed filter position.) | |
| | Aperture iris diaphr. | Fully opened. | Opened to 70 – 80% of the objective N.A. | | |
| DIC attachment | Nomarski prism | OUT | | IN<br>(Contrast is controlled by movement of Nomarski prism.) | OUT |
| | Analyzer | OUT | | IN | |
| Field iris diaphragm | | Opened so as to circumscribe the field of view. | | | |
| B & W film | Lamp voltage | 6 – 12V | | | |
| | Filter | 43-IF550-W45 | | | |
| Color film (daylight type) | Lamp voltage | 8 – 9V | | | |
| | Filter | 45-LBD2-N, 45-ND6-W45 | | | |
| Plastic vessel | | OK | | NO | |

(Cut off this page at dotted line and put it on the wall near the microscope for use as a reminder of microscopic procedure.)

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## 8–2 Observation

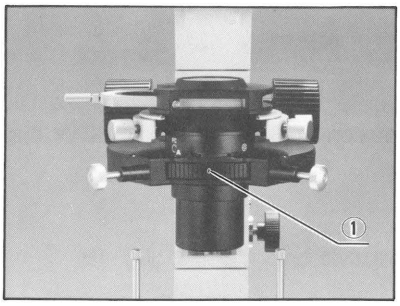


Fig. 13

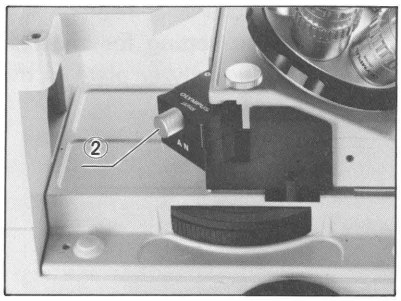


Fig. 14

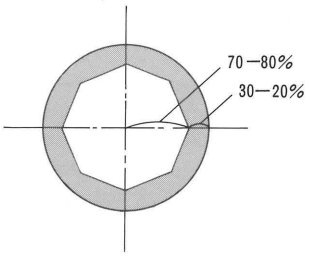


Fig. 15

### 1. Method of Differential Interference Contrast Observation in Detail

★ **Make sure that the procedure for the condenser centration (page 5) and polarizer adjustment (page 6) have been completed.**

1) Rotate the condenser turret ① until the turret is matched with the objective magnification. (Fig. 13)

2) Choose a most suitable contrast color in a range from 0 order (black) to second order (blue) (or 0 to 700 nm) by rotating the prism adjustment knob ②. (Fig. 14)

| Contrast color in background | Effect |
|---|---|
| Dark | Observation similar to darkfield. |
| Gray | Visual sensitivity to detect small path difference as relief-like image becomes most pronounced. |
| Magenta | A change in path difference can be most sensitively detected as a change of color. |

★ **Take care to keep the surface of the specimen as clean as possible because the surface to observe may be marked by surface contamination. Even a small amout of contamination may be shown up because of extreme sensitivity in differential color contrast.**

3) 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.

4) In order to achieve optimum objective performance, the opening of the field iris diaphragm should circumscribe the field of view. It is often preferable, however, to stop down the aperture iris diaphragm by about 70 to 80% of the objective N.A. (Fig. 15)

### 2. Image Magnification for Observation and Photomicrography

[Observation] Objective mag. X Eyepiece mag.

[Photomicrography]

35mm format: Objective mag. X NFK photo eyepiece mag.
Large format: Objective mag. X NFK photo eyepiece mag. X3

3. When using this microscope for Nomarski differential interference contrast and fluorescence observation at the same time, the analyzer of the DIC attachment will reduce the light intensity and result in darkening of the fluorescence image.
Before using such an observation method, follow the next procedures:

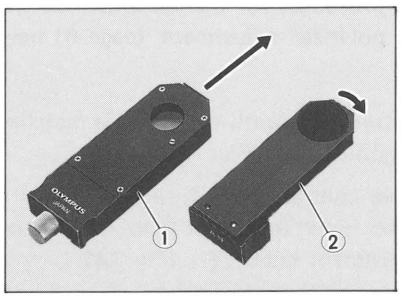


Fig. 16

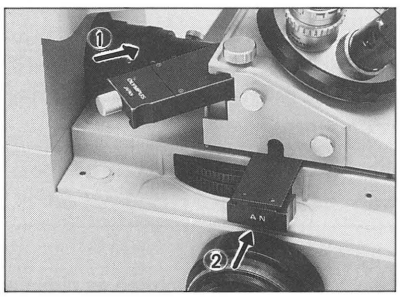


Fig. 17

1) Push out the analyzer plate ② to remove it from the DIC attachment ① in the direction as shown by the ARROW. (Fig. 16)

2) Rotate the analyzer clockwise until it stops. (Fig. 16)

3) Reattach the DIC attachment ① that was removed the analyzer plate to its original position.
Insert the analyzer plate ② into the exciter filter insertion slot.
★ **Verify that the vibration position setting for analyzer plate is in NA before uniting the analyzer plate to the DIC attachment.**

# 9 TROUBLESHOOTING GUIDE

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

| Trouble | Cause | Remedy |
|---|---|---|
| a) No interference color can be seen. | Polarizer is not in the light path. | Insert the polarizer into the light path. (page 6) |
| | The phase contrast turret is not correctly aligned. | Align the turret correctly. (pages 5, 6) |
| | The Nomarski prism is not in the light path. | Insert the DIC attachment into the light path. (page 4) |
| b) The interference color can be seen irregularly, or its contrast is low. | The condenser height is not correctly adjusted. | Bring the condenser into focusing position accurately. (page 5) |
| | The objective magnification and the turret are not matched. | Match the objective magnification and the turret correctly. (page 3) |
| | Designated objective is not used. | Use the designated objective (e.g. S Plan 10X, LWDCD Plan 20X, 40X). (ULWD series objectives are incompatible.) (page 4) |
| | Polarizer is not set in correct position. | Correct the direction of polarizer vibration in conjunction with analyzer. (page 6) |
| | The vessel has polarization. (Generally, plastic vessels are incompatible.) | Use a vessel of non-polarization. (2nd cover, page 7) |
| c) Field of view is partially cut off. | DIC attachment is stopped midway. | Insert it completely until it stops. (page 4) |
| d) The light annulus and phase annulus are not matched. | No designated phase objective is used. | Use designated phase objectives. (page 1) |
| e) Image is not attained for crossed NICOL. | Vibration position setting for analyzer plate is not in 'NA'. | Set vibration position setting in 'NA'. |

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