

Nikon

**DIASCOPIIC DIC
NOMARSKI
ATTACHMENT
TMD-NT**

**for Inverted Microscope DIAPHOT-TMD
Instructions**

NIPPON KOGAKU K.K.

Thank you for your purchasing the Nikon DIASCOPIIC DIC NOMARSKI ATTACHMENT. The DIASCOPIIC DIC NOMARSKI ATTACHMENT permits us to perform phase contrast (with 10× and 20× objectives) microscopy as well as differential interference microscopy using the Inverted Microscope DIAPHOT-TMD.

Before using the attachment, read through this instructions and also that of the Inverted Microscope DIAPHOT-TMD carefully.

CAUTIONS

- 1 Strain-free glasses**
The optical elements of this attachment being constructed of strain-free glasses, take particular caution in handling the objectives and condenser lenses not to cause strain to them.
- 2 Environment**
Avoid the use of the microscope in a dusty place, where it is subject to vibrations or exposed to high temperatures, moisture or direct sunlight.
- 3 Cleanliness of lenses**
Do not leave dust, dirt or finger marks on the lens surfaces.
- 4 Glass slides and coverglasses**
For preparing specimens, use glass slides and coverglasses both free from strain and dust.

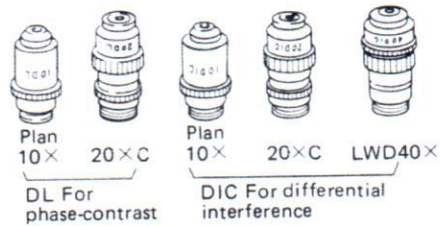
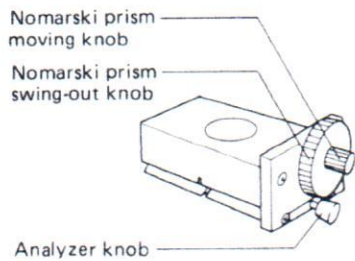
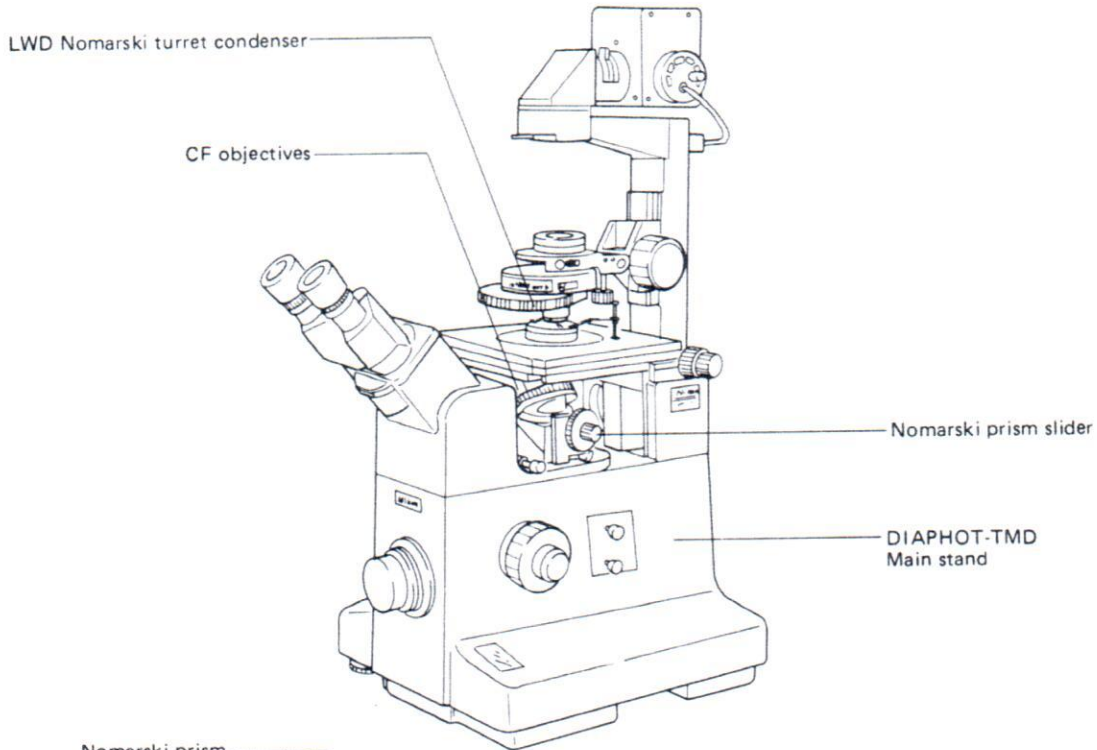
CARE AND MAINTENANCE

- 1 Lens cleaning**
To clean the lens surfaces, remove dust using a soft brush or gauze. Only for removing finger marks or grease should soft cotton cloth, lens tissue or gauze lightly moistened with absolute alcohol (methanol or ethanol) be used. For cleaning the objectives or removing immersion oil, use xylene.
- 2 Other cleaning**
Avoid the use of the any organic solvent (for example, thinner, ether, alcohol, xylene, etc.) for cleaning the painted surfaces and plastic parts of the instrument.
Observe sufficient caution in handling methanol and xylene.
- 3 Dismantling**
Never attempt to dismantle the instrument so as to avoid the possibility of impairing the operational efficiency and accuracy.
- 4 Storage**
When not in use, store the attachment in a place free from moisture and fungus. It is especially recommended to keep the objectives and turret condenser in an air-tight container containing desiccant.
- 5 Periodical check**
To maintain the performance of the instrument, we recommend to check the instrument periodically.
(For details of this check, contact your dealer.)

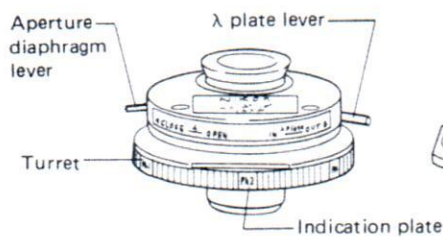
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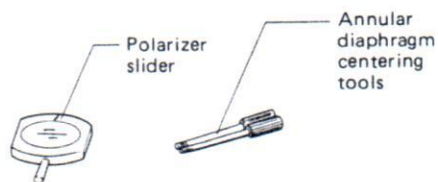
I. NOMENCLATURE



Nomarski prism slider



CF objectives



LWD Nomarski turret condenser

II. ASSEMBLY

1. Attaching the LWD Nomarski turret condenser

- ① If the LWD or the Extra LWD phase-contrast turret condenser has been already attached to the condenser mount, remove it beforehand.
- ② Attach the LWD Nomarski turret condenser to the condenser mount and clamp the screw. (Fig. 2)

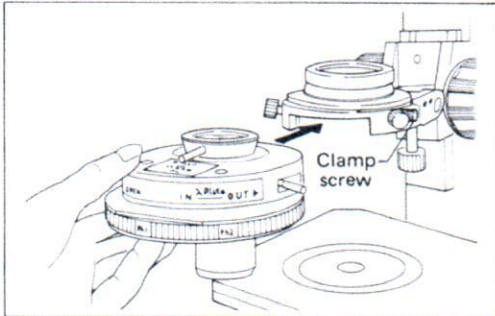


Fig. 2

2. Attaching the Nomarski prism slider

- ① Remove the covers from the right and left sides under the nosepiece mount.
- ② Insert the Nomarski prism slider as shown in Fig. 3.
Make certain that it positively click-stops.

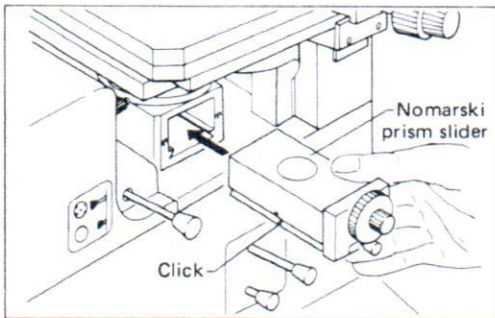


Fig. 3

3. Mounting the objectives

Beforehand, rotate the coarse focus knob to move the revolving nosepiece to the lowest position.

Attach the objectives to the nosepiece from the left side one after another in such positions as shown in Fig. 4, when the nosepiece is viewed from above.

Be careful not to let the tops of objective touch with the stage, etc.

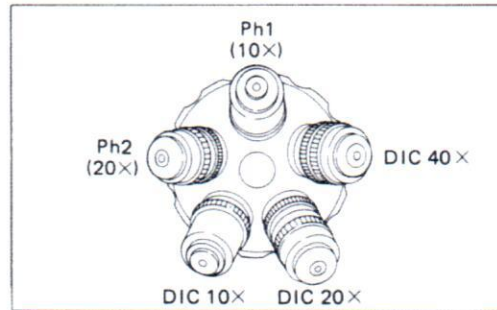


Fig. 4

For assembling the other parts, refer to p.6~p.9 of Instructions, "DIAPHOT-TMD".

III. PREPARATION

To take full advantage of the attachment, perform the adjustments as below, for centering, exact Crossed Nicols and sharp border lines of the field diaphragm image.

1. Switching ON the power source, and placing the specimen

- ① Connect the power source cord to the socket.
- ② Turn ON the power switch, and set the brightness indicator to 6.
- ③ Place the specimen onto the stage. Fasten it in position using the specimen clips, if necessary.

2. Adjusting the interpupillary distance

- ① Set the condenser turret to [Ph1], and the observation turret to [O].
- ② Turn the field diaphragm control lever to [OPEN] to fully open the diaphragm.
- ③ Bring the specimen image into focus, using the 10× objective.

Adjust the interpupillary distance, as shown in Fig. 5, so that the right- and left eye viewfields come together into coincidence.

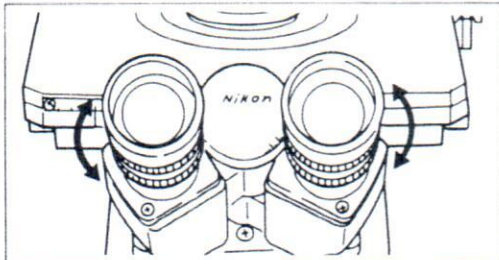


Fig. 5

3. Diopter adjustment

- ① Pull the photo mask sliding knob up to the limit to bring the photo mask into the optical path.
- ② Turning the diopter ring on each eyepiece, until the crossline image appears sharp. Do this adjustment for right- and lefthand eyepieces.

※The CF eyepieces being of high eyepoint type, when the observer uses his eyeglasses, it will not be necessary to remove but only to bend the rubber eyeguards. (Fig. 6)

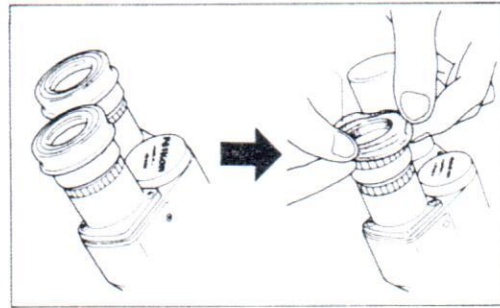


Fig. 6

4. Centering the turret condenser

- ① Place the specimen on the stage.
- ② Pull the analyzer knob to bring the analyzer out of the optical path.
- ③ Set the turret condenser to [O], and the observation turret of the Microscope TMD to [O].
- ④ Swing the objective 10× into the optical path, and focus on specimen.
- ⑤ Manipulate the field diaphragm control lever of the microscope to close down the diaphragm to its smallest limit. Rotate the condenser focus knob to move the condenser vertically so that a sharp image of the field diaphragm is formed on the specimen surface.
- ⑥ Bring the field diaphragm image to the center of the field of view by means of the condenser centering screws. (Fig. 7-1)

- ⑦ Change over to the objective $40\times$, and adjust the field diaphragm so that the image of the diaphragm is about the same as that of the field of view, as shown in Fig. 7-2.

If not centered, use the condenser centering screws again.

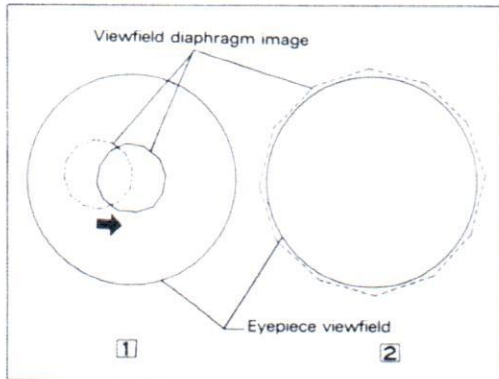


Fig. 7

5. Centering the lamp

- ① Set the condenser turret to [O]. Set the observation turret to [O].
- ② Fully open the viewfield and the aperture diaphragms.
- ③ Using the $20\times$ objective, bring the specimen image into focus.
- ④ Change over the observation turret to [B]. Turn the Bertrand lens focus lever to bring the phase-contrast ring inside the objective into focus. (Fig. 8)

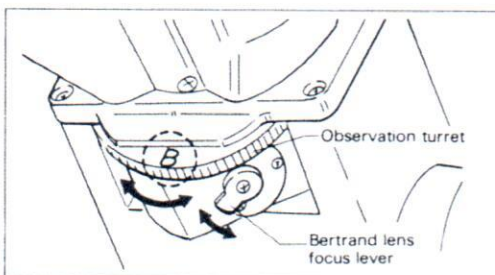


Fig. 8

- ⑤ Remove the diffuser from the filter holder. Releasing the lamp housing clamp screw, move the housing back and forth to have the lamp filament image focused on the phase-contrast ring. (Fig. 9)

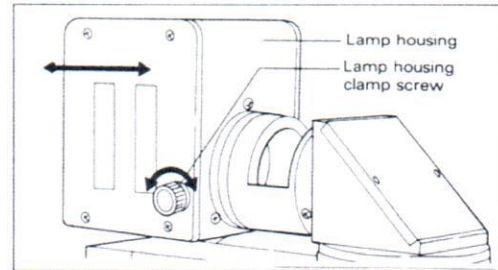


Fig. 9

- ⑥ After releasing the socket sleeve clamp screw, as shown in Fig. 10, manipulate the lateral centering screw and vertical centering ring to bring the filament image to the center, as shown in Fig. 11.

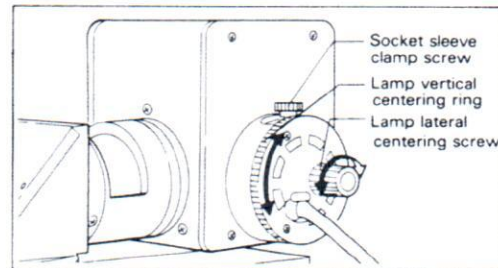


Fig. 10

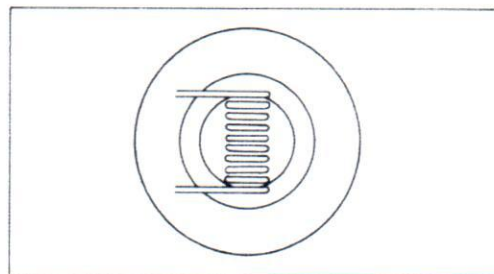


Fig. 11

- ⑦ After finishing the above lamp centering procedure, drop the diffuser with its mat surface faced toward the user into the closest groove of the filter holder.

6. Adjustment of the vibration direction of light

- ① Be sure that the white line on the polarizer slider faces upward. (Fig. 12)

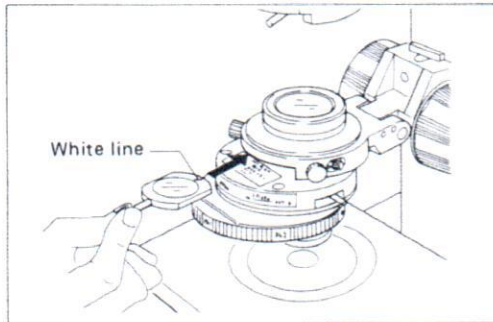


Fig. 12

- ② Turning the aperture diaphragm lever, fully open the condenser aperture diaphragm, and turning the λ plate lever, bring the λ plate out of the optical path.
- ③ Be sure of the [O] setting on the turret of the condenser.
- ④ Fasten the condenser swing-out clamp.
- ⑤ Push the analyzer into the optical path, and bring the Nomarski prism out of the optical path by turning the Nomarski prism swing-out knob.
- ⑥ Swing the objective DIC40 \times into the optical path.
- ⑦ Remove the specimen out of the optical path.
- ⑧ Turning the observation turret, set the indication [B].
Focus on the exit pupil of objective by turning the Bertrand lens focus lever.
- ⑨ Slightly release the clamp screw of the condenser and rotate the condenser to a little degree as shown in Fig. 13, until a dark cross image, as shown in Fig. 14, appears, and fasten up the condenser clamp screw.

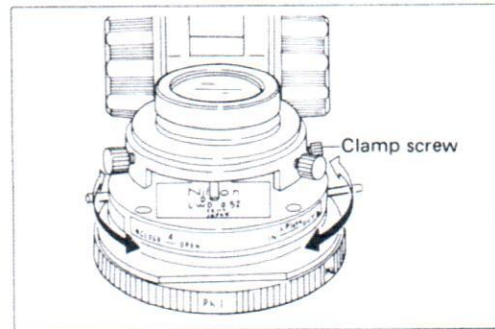


Fig. 13

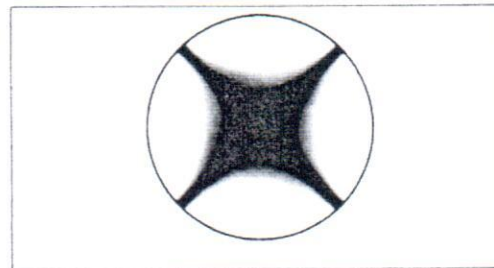


Fig. 14

Since the appearance of such a cross image which proves the correct positioning of the polarizer and analyzer for Crossed Nicols is important for efficient use of the microscope, the above procedure should be carefully and positively conducted.

7. Centering the annular diaphragm for phase contrast microscopy

- ① Pull the knob to put the analyzer out of the optical path.
- ② Place the specimen on the stage, and swing in the objective Ph1 (10×).
- ③ After making sure of the full opening of the aperture diaphragm, set the condenser turret to [Ph1].
- ④ Turn the observation turret to [O], and focus on the specimen.
- ⑤ Set the observation turret to [B], and turn the Bertrand lens focus lever to bring the image of the phase plate in the objective into sharp focus.
- ⑥ If the image of the condenser annular diaphragm is not found coincided to that of the phase plate in the objective, make adjustment, manipulating the centering tools, as shown in Fig. 15, and Fig. 16.

Such coincidence is to be exactly attained, otherwise a remarkably low image contrast of phase difference would result.

- ⑦ Change over the objective to Ph2 (20×), and revolve the turret to [Ph2], thereafter repeat the above centering procedure.

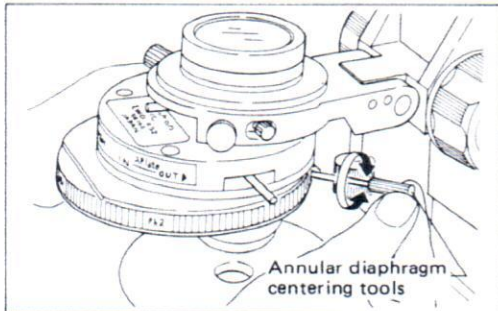


Fig. 15

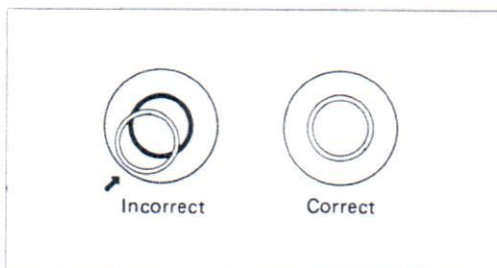


Fig. 16

IV. MICROSCOPY

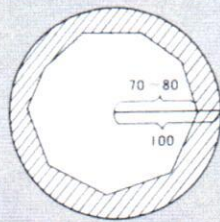
1. Differential interference microscopy procedure

1 Place the polarizer, analyzer and objective-side Nomarski prism into the optical path. Put out the λ plate.

2 Swing in the DIC objective to be used to the optical path.
Set the position of the condenser turret to the power of the objective.

3 Focus on specimen.

4 Manipulate the aperture diaphragm lever to close down the aperture diaphragm. When it is stopped down to 70~80% of the numerical aperture of the objective, a good image of appropriate contrast will be obtained. (Fig. 17)



Size of the aperture diaphragm **Fig. 17**

(The closed diaphragm can be seen on the exit pupil inside the objective with the observation turret set to [B].)

5 When observing a specimen of low phase difference, for achieving the best contrast, move the objective-side Nomarski prism by rotating its moving knob, thus changing the background to a sensitive color of grey.

6 After placing the λ plate into the optical path, rotate the Nomarski prism moving knob further on, to change the background to a sensitive color of red-violet, and the specimen with a difference of refractive index or thickness will show up different interference colors corresponding to the slope of refractive index or thickness, producing the highest color contrast.

7 For a specimen of relatively large phase difference, place the λ plate into the optical path, and rotate the Nomarski prism moving knob to the position where the best contrast image is obtained.
(The interference color can be changed continuously from yellow to red-violet, up to blue.)

8 The use of a green interference filter will serve to achieve a better contrast image.

Note:

- For each objective, a different condenser-side Nomarski prism being equipped in the condenser turret, it is necessary to change-over the turret setting, when using another objective magnification.
- The seeing of the differential interference image is directional, and in this attachment the shearing is realized only in the lateral direction.
So the border surface in the longitudinal direction, for instance, affords the highest detecting sensitivity, and in the direction at right angles to that, the lowest.
Turn the specimen on the stage to find out the position where the highest contrast image of the specimen is obtained.
- Differential interference microscopy is impossible when a culture dish made of plastic is used, because it affects the effect of differential interference.
Use a culture dish which is made of glass with good flatness.

2. Phase contrast microscopy procedure

- 1 Bring the λ plate, the objective-side Nomarski prism and the analyzer out of the optical path.
- 2 Remove the polarizer slider from the condenser.
- 3 Push a green interference filter into the filter frame, and place this into the filter holder in front of the lamp housing of the microscope.
- 4 Manipulate the aperture diaphragm control lever to fully open the diaphragm.
- 5 Swing the objective to be used, Ph1 (10 \times) or Ph2 (20 \times), into the optical path.
- 6 Set the turret of the condenser to [Ph1] or [Ph2].

The above procedure for phase contrast microscopy, serving for comparing the phase contrast with the differential interference method, will be utilized as a finder to find out the target on the specimen.

3. Brightfield microscopy procedure

- 1 Bring the λ plate, the objective-side Nomarski prism and the analyzer out of the optical path.
- 2 Remove the polarizer slider.
- 3 Set the condenser turret to [O].
- 4 Swing in the objective to be used.

The above procedure for brightfield microscopy, will facilitate finding out the target on the specimen. Furthermore, closing down the aperture diaphragm at this time is recommended for easier viewing.

V. PHOTOMICROGRAPHY

For photomicrography, refer to the instructions of DIAPHOT-TMD.

VI. TROUBLE SHOOTING TABLE

Although nowhere you can find any disorder or derangement in the attachment, if you encounter some difficulty or dissatisfaction, recheck the use, referring to the table below, and also referring "VII. TROUBLE SHOOTING TABLE" in the instructions of DIAPHOT-TMD.

Failures	Causes	Actions
Shading of the viewfield	<ul style="list-style-type: none"> Turret of the condenser in halfway position Objective-side Nomarski prism in halfway position Analyzer in halfway position Nomarski prism slider in halfway position 	<ul style="list-style-type: none"> Turn it to click-stop position Swing it positively Push or pull it positively Insert it positively
No interference color obtained by differential interference method	<ul style="list-style-type: none"> Plastic culture dish used Polarizer not in optical path Turret of condenser not correctly set Objective-side Nomarski prim not in optical path Analyzer not in optical path 	<ul style="list-style-type: none"> Used glass culture dish with good flatness Push it with its white line faced upward Set the same indication with that of objective power Swing it into the optical path Push it into optical path
Not uniform interference colors, even though appear	<ul style="list-style-type: none"> Not correct height of condenser Not correct setting of objective magnification on turret Insufficient "Crossed Nicols" setting Dust on lens (Objective, condenser, specimen) Polarizer placed up side down 	<ul style="list-style-type: none"> Move condenser vertically to get field diaphragm image Correct setting Perform "6. in III. PREPARATION" Thorough cleaning (Take special caution against dust, on account of polarization type of the interference microscope) Place it with its white line faced upward
Low contrast or no effect by phase contrast method	<ul style="list-style-type: none"> Insufficient centering of phase contrast diaphragm and phase plate Aperture diaphragm too much closed Ph number of objective and that of condenser turret not coincided 	<ul style="list-style-type: none"> Follow the procedure of "7. in III. PREPARATION" Fully open the diaphragm Set the same Ph number of condenser turret with that of objective

We reserve the right to make such alterations in design as we may consider necessary in the light of experience. For this reason, particulars and illustrations in this handbook may not conform in every detail to models in current production.



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