Nikon

BIOPHOT

INSTRUCTIONS

NIPPON KOGAKU K.K.

CAUTIONS

1) When carrying the microscope, remove the lamp housing and hold the instrument with one hand inserted beneath the front of the base and the other supporting the lower portion of the upright arm.
   The instrument weighs about 22 kg. (48 lbs.)
   Never hold the instrument by its horizontal arm for carrying or moving.
2) Before attaching and detaching the arm, be sure that the revolving nosepiece has been removed and the stage surface sufficiently lowered.
   When attaching and detaching the revolving nosepiece, observe the cautions given on pages 9 and 14.
3) When looking into the eyepieces at the same time the filters built-in the microscope base are changed, the field of view may become excessively bright.
4) Handle the microscope gently, taking care to avoid sharp knocks.
5) 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.
6) Do not leave dust, dirt or finger marks on the lens surfaces.
7) Be certain that the power source voltage setting on the input voltage changer at the side of the microscope base is correct.
8) Before replacing the fuse disconnect the plug from the power source.

CARE AND MAINTENANCE

1) To clean the lens surfaces, remove dust using a soft hair brush or gauze. Only for removing finger marks or grease, should soft cotton cloth, lens tissue or gauze lightly moistened with xylene, alcohol, ether, etc. be used.
   For cleaning the objectives only use xylene.
2) Avoid the use of any organic solvent for cleaning the painted surfaces and plastic parts of the instrument.
3) Never attempt to dismantle the instrument so as to avoid the possibility of impairing the operational efficiency and accuracy.
4) When not in use, cover the instrument with the accessory vinyl cover, and store it in a place free from moisture. It is especially recommended that the objectives and eyepieces be kept in an air-tight container containing desiccant.

CONTNETS

I. NOMENCLATURE

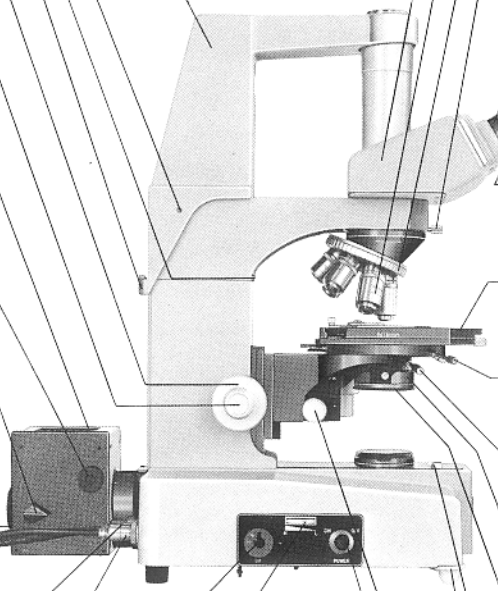


Photo support bracket
Attached for photomicrography

Plastic screw

Arm stop

Photo support bracket lock screw

Coarse focus knob
One rotation for stage movement of 24mm

Fine focus knob
One rotation for 0.2mm, one division 0.002mm

Lamp housing
Contains halogen lamp 12V 100W

Collector lens focusing knob
Rotation brings the lamp filament image into focus.

Diffuser lever
Turning toward the operator brings the diffuser into the optical path. Usually use it in this position for routine work.

Rear mirror
Back and forth movement permits focusing the light reflected by the mirror.

Power output socket
Max. output 12V 100W

Power input plug

Input voltage changer
Insert a screwdriver and rotate the changer, while pressing in, to adjust the input voltage. For replacing the fuse, rotate the changer to the key groove and the fuse will be removed by spring action.

Brightness control dial
Turning toward H will increase the brightness of illumination.

Trinocular eyepiece tube

Objective
Refer to p. 15

Revolving nosepiece
Accepts 6 objectives, includes filter slide.

Revolving nosepiece clamp screw
Serves to fasten nosepiece. Refer to p. 14

Diopter ring
For eyesight adjustment

Eyepiece
When not using spectacles, extend the eyeguards.

Large-type mechanical stage
Travel ranges 52mm x 76mm

Stage centering tools
For centering stage rotation center to optical axis.

Condenser centering screws
For centering field diaphragm

N.A. indicator scale

Condenser aperture diaphragm ring
For adjustment of aperture of condenser refer to p. 13.

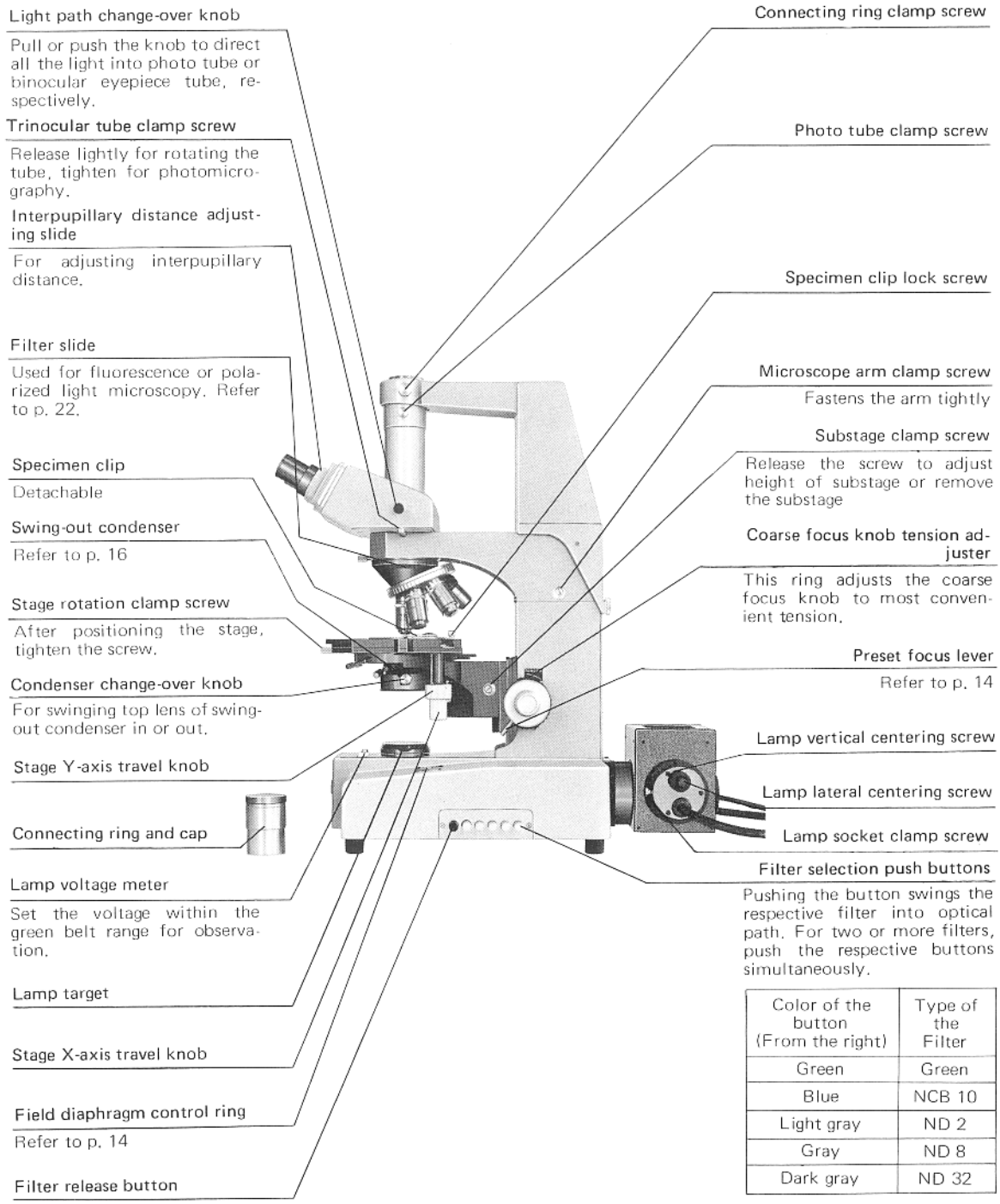
Power indicator light

Horizontal positioning screw
To stabilize microscope on the desk in the horizontal position

Knob for condenser focus

Main power switch

Fig 1. Model VBS



Color of the button (From the right)	Type of the Filter
Green	Green
Blue	NCB 10
Light gray	ND 2
Gray	ND 8
Dark gray	ND 32

Fig. 2 Model VBS

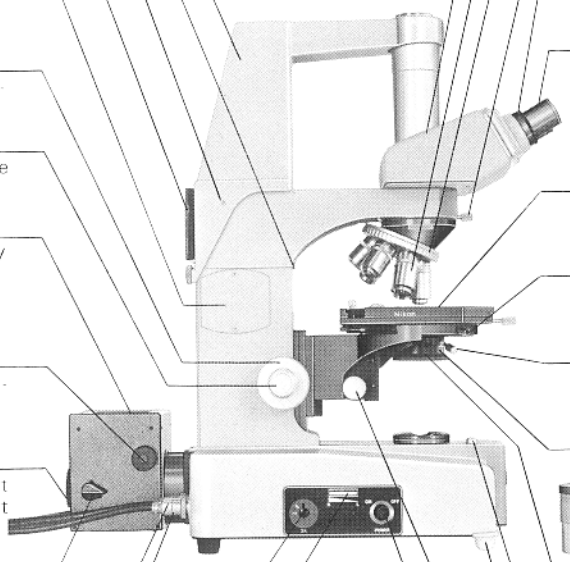


Photo support bracket
Attached for photomicrography

Arm stop

Plastic screw

Dovetail for attaching high-intensity light source
The mercury lamp, for example, is attached to this dovetail.

Filter cassette port
Remove the cover and attach the diascopic fluorescence excitation filter cassette to this part.

Coarse focus knob
One rotation for stage movement of 24mm

Fine focus knob
One rotation for 0.2mm, one division 0.002mm

Lamp housing
Contains halogen lamp 12V 100W

Collector lens focusing knob
Rotation brings the lamp filament image into focus.

Rear mirror
Back and forth movement permits focusing the light reflected by the mirror.

Diffuser lever on lamp housing
Turning toward the operator brings the diffuser into the optical path. Usually use it in this position for routine work.

Power output socket
Max. output 12V 100W

Power input plug

Input voltage changer
Insert a screwdriver and rotate the changer, while pressing in, to adjust the input voltage. For replacing the fuse, rotate the changer to the key groove and the fuse will be removed by spring action.

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Condenser aperture diaphragm ring
For adjustment of aperture of condenser refer to p. 13.

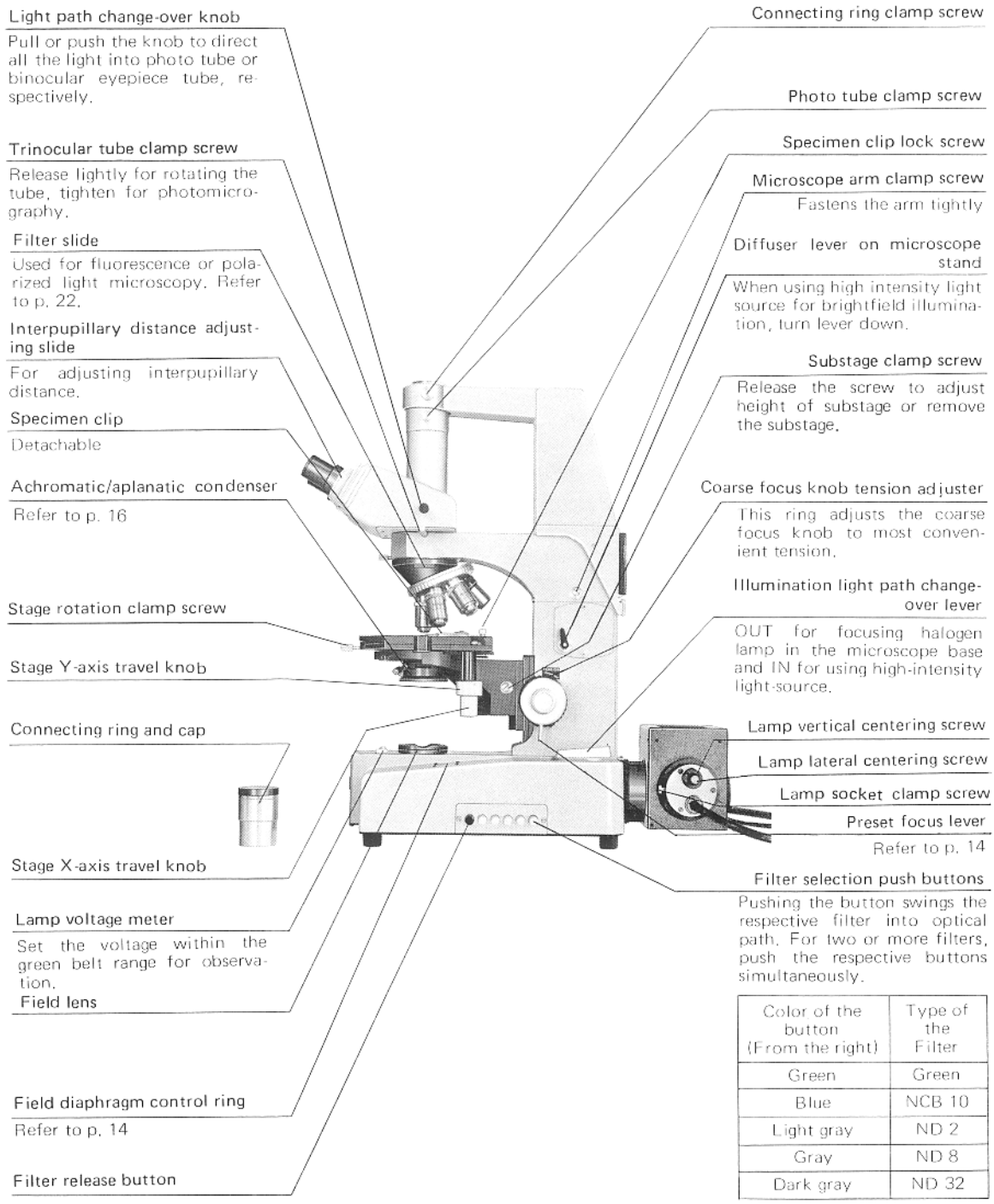
Power indicator light

Horizontal positioning screw
To stabilize microscope on the desk in the horizontal position

Knob for condenser focus

Main power switch

Fig. 3 Model VBD



Color of the button (From the right)	Type of the Filter
Green	Green
Blue	NCB 10
Light gray	ND 2
Gray	ND 8
Dark gray	ND 32

Fig. 4 Model VBD

II. ASSEMBLY

1. Attaching the lamp housing and halogen lamp

1) Remove the cover from the dovetail on the base of microscope, and slide-mount the lamp housing onto the base dovetail fitting. Take care not to tilt the lamp housing.
2) Detach the bulb socket. When inserting the halogen bulb into the socket, do not remove the bulb cover to avoid the possibility of leaving any fingermarks on the envelope. After attaching the lamp, remove the cover.
3) Thereafter, insert the socket into the lamp housing as far as it goes and fasten firmly with the clamp screw.
4) Connect the plug of the cord from the clamp to the output receptacle on the microscope base.
5) Connect the input cord plug to the input receptacle on the microscope base, and fasten it firmly with the lock ring.

2. Attaching the substage and stage

1) Slide the substage condenser holder on the dovetail fitting. Attach in such a position that the top end of the substage is at the level shown in Fig. 5. Fasten it firmly in this position with the clamp screw, using a 5mm screwdriver.

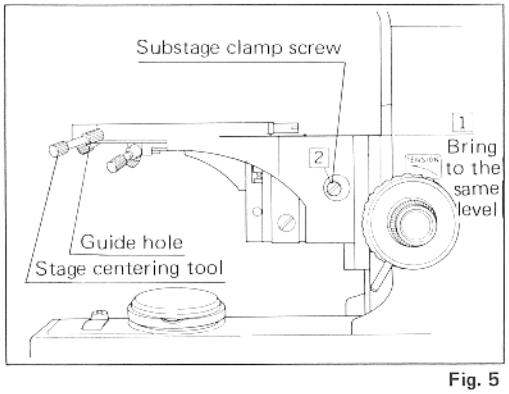


Fig. 5

2) Place the stage centering tools into the guide holes and turn clockwise (Apply no force!), until the protrusions fit into the slits on the centering screws inside.

Thereupon, loosen the screws sufficiently by counterclockwise turn.

3) Turn the circular dovetail on the underside of the stage to a position where the notch on the dovetail faces the opposite side of the stage rotation clamp screw, as shown in Fig. 6. Tighten the clamp screw firmly.

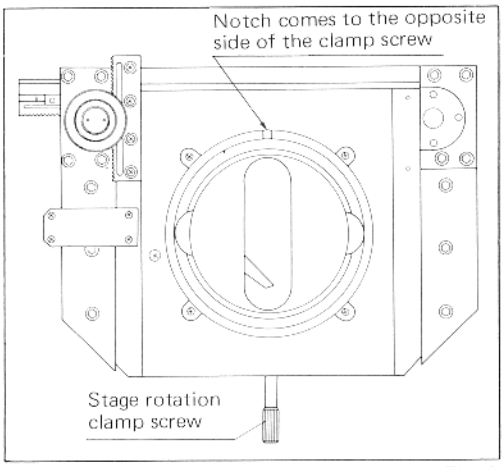


Fig. 6

4) Bring the notch on the circular dovetail into coincidence with the pin on the substage, tilt the stage as shown in Fig. 7-[1]. Then, press the circular dovetail against the centering spring, as shown in Fig. 7-[2] mount the substage in the horizontal position.

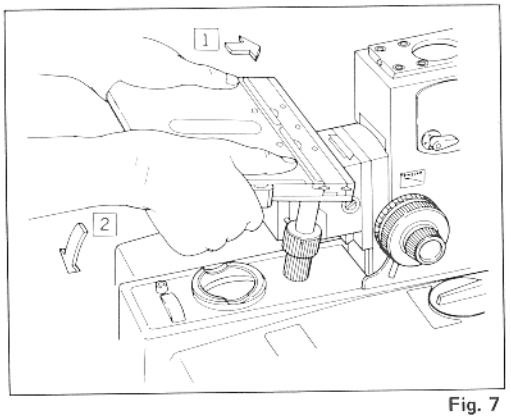


Fig. 7

5) Attach the two specimen clips onto the top surface of the stage with the two lock screws.

3. Attaching the microscope arm

1) Release the arm clamp screw and carefully slide the arm into the dovetail on the top surface of the microscope stand as far as it will go.
2) Fasten the arm lock screw firmly, using a 5 mm screwdriver.

4. Attaching the Trinocular tube

1) Slacken the trinocular tube clamp screw sufficiently.
2) Tilt the tube as shown in Fig. 8. Push it toward the clamp screw, seat it firmly in a horizontal position.

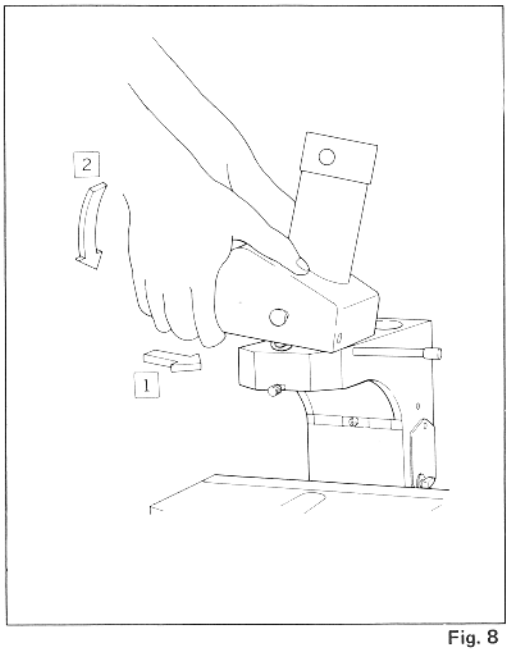


Fig. 8

3) Tighten the trinocular tube clamp screw.
4) Put the CF eyepieces into the binocular eyepiece sleeves.

5. Attaching the photo support bracket

1) Remove the connecting ring from the trinocular eyepiece tube.
2) Mount the photo support bracket over the high-intensity light-source attaching dovetail, as shown in Fig. 9, fasten it onto the back of the microscope stand by means of the photo support bracket lock screw.

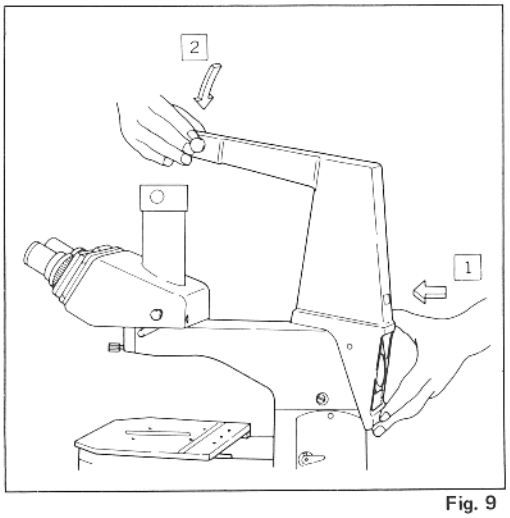


Fig. 9

3) Fasten the photo support bracket with the plastic screw on each side of the support, so that it no longer moves laterally.
4) Insert the CF photo eyepiece into the vertical photo tube.

Note: The connecting ring provided on top of the trinocular eyepiece is to be used when the accessory photo support bracket is not employed. (e.g. for photomicrography using the 15X eyepiece or image projection using the projection screen).

Use the photo support bracket in all cases where photographic results are especially critical.

6. Attaching the Microflex

Unscrew the connecting ring from the bottom of the photomicrographic attachment, such as Nikon Microflex HFM.

In its place, attach the connecting ring belonging to the photo support bracket. Mount the Microflex HFM and fasten it with the clamp screw. (The use of the connecting ring of the HFM is necessary for mounting on microscopes other than the Nikon V-series).

For assembling the Microflex HFM and other attachments, refer to their respective instruction manuals.

7. Attaching the objectives and revolving nosepiece

1) Mount the objectives on the nosepiece, as shown in Fig. 10, in such positions that, when viewed from above, their magnifying power increases, as the nosepiece is revolved clockwise.

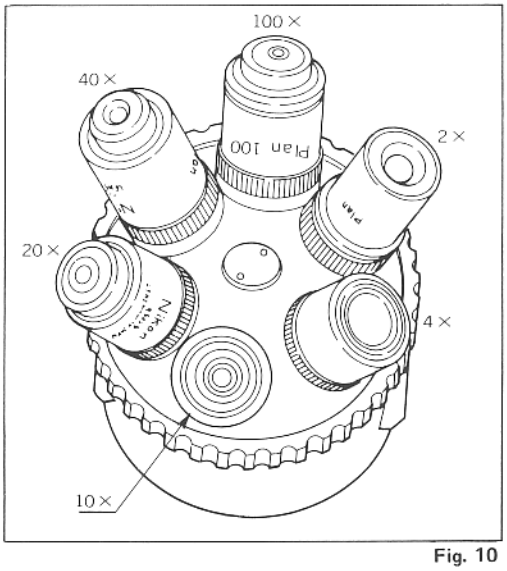


Fig. 10

2) Thereupon, lower the stage and tilt the nosepiece with the objectives attached, and press it against the clamp screw, as shown in Fig. 11, seat it to the microscope stand in the horizontal position.

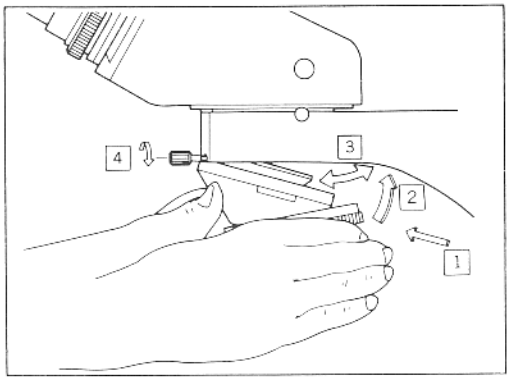


Fig. 11

3) Revolve the nosepiece as a whole to have its positioning pin settle into the groove and lock into place with the clamp screw.

III. PREPARATION

1. Centering the lamp

1) Place the A.C. power cord plug into the input receptacle and turn the power switch on. Turn the brightness control dial to set the lamp voltage to 7-8 on the voltmeter.
2) Manipulate the coarse focus knob to raise the stage until it nearly touches the objective.
3) Lower the condenser carrier, and insert the lamp target into the condenser dovetail until it reaches its innermost limit.
4) Turn the condenser focus knob to lower the condenser carrier so that the lock screw is at the same level as the bottom surface of the substage, as shown in Fig. 12.

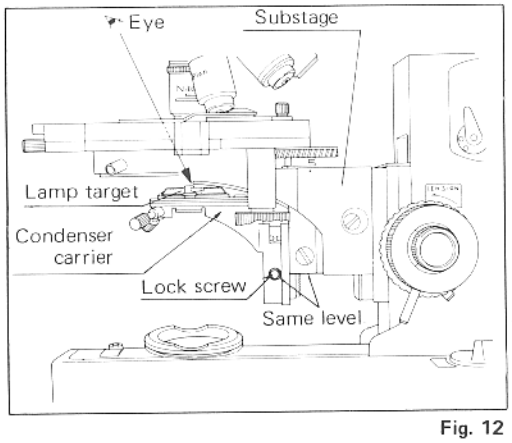


Fig. 12

5) Turn the diffuser lever on the lamp housing forward to swing the diffuser (lemon skin filter) out of the optical path. Manipulate the focus knob of collector lens to form a sharp image of the lamp filament on the target.
6) Manipulate the lamp centering screws to center the filament image on the crosslines of the lamp target as shown in Fig. 13.

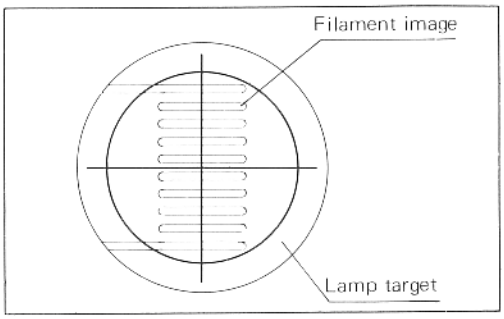


Fig. 13

7) Focus the mirror at the rear of the lamp housing back and forth, to superimpose the slightly darker image of the lamp filament on the brighter direct image. It may be necessary to manipulate the centering screws again to achieve alignment. (See Fig. 14)

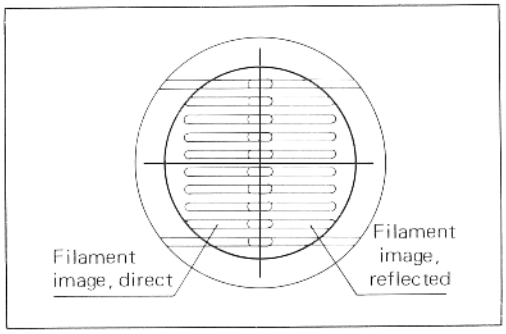


Fig. 14

8) Turn the diffuser lever on the lamp housing toward the operator to bring the diffuser into the optical path.
9) Remove the lamp target from the condenser dovetail.

2. Mounting the condenser

1) Rotate the condenser focus knob to lower the condenser carrier to its lowest limit.
2) Slide the condenser into the dovetail fitting with the N.A scale of the condenser facing the operator.
3) Fasten the clamp lever. Turn the condenser knob to raise the carrier to the highest limit.

3. Adjusting the interpupillary distance and diopter correction.

1) Push in two filter selection buttons simultaneously, one dark gray for ND 32 filter and one blue for NCB 10 filter.
2) Holding the right and left hand milled rings at the base of the binocular eyepiece sleeves, as shown in Fig. 15, and looking into the eyepieces, adjust the interpupillary distance, so that both the right and left viewfields become one. Look at the scale and remember this distance reading for future use.

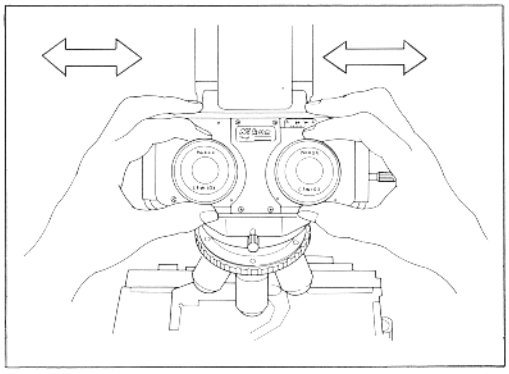


Fig. 15

3) Place a specimen on the stage. Using the 10X objective, look into the righthand eyepiece and manipulate the focus knobs (coarse and fine) to sharply focus the image of the specimen.
4) Then, look into the other eyepiece. If the image is defocused, rotate the diopter adjusting ring to focus it sharply.
5) The CF eyepiece being of high eyepoint type can be used with spectacles. When not wearing spectacles, extend each eyeguard as shown in Fig. 16, to a convenient position.

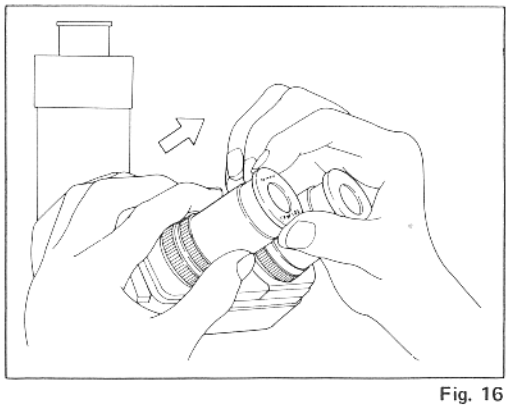


Fig. 16

4. Centering the condenser lens

1) Close the field diaphragm in the microscope base to its smallest size by means of the field diaphragm control ring. Rotate the condenser knob to move the condenser vertically so that a sharp image of the field diaphragm is formed on the specimen surface.
2) Close the field diaphragm and focus it sharply in the center of the field of view by means of the condenser centering screws.
3) Change over to the 40X objective, 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. 17. If not centered, use the condenser centering screws again.

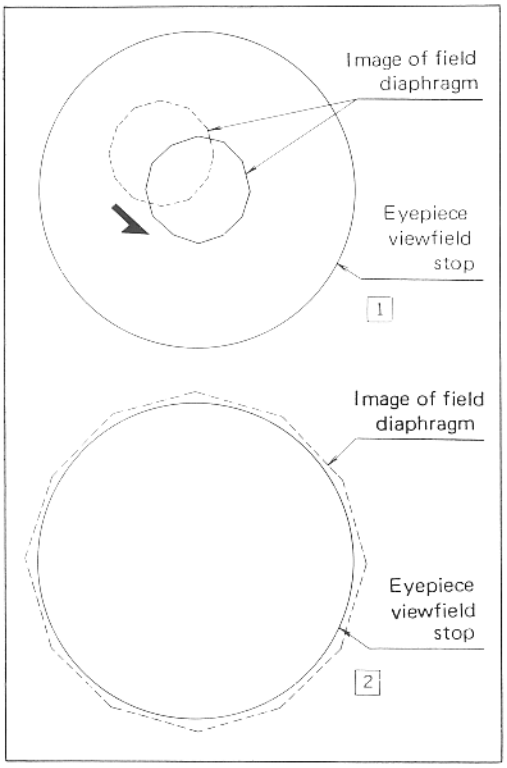


Fig. 17

5. Centering the stage

For framing and composing in photomicrography, stage centration may be accomplished as follows:

1) Using the 10X objective choose an appropriate target in the specimen. Manipulate the stage cross-travel knobs to move the target to the center of the field of view.

2) Release the stage rotation clamp screw and rotate the stage about 180°. If the target is displaced from the center of the field of view, move it one half of the displacement by means of the stage centering tools.

(Fig. 18)

(Note that since the lefthand cross-travel knobs are not rotatable over 90° counter-clockwise, some estimation will be necessary.)

3) Recenter the target chosen to the exact center of the rotation by manipulating the stage cross-travel knobs.

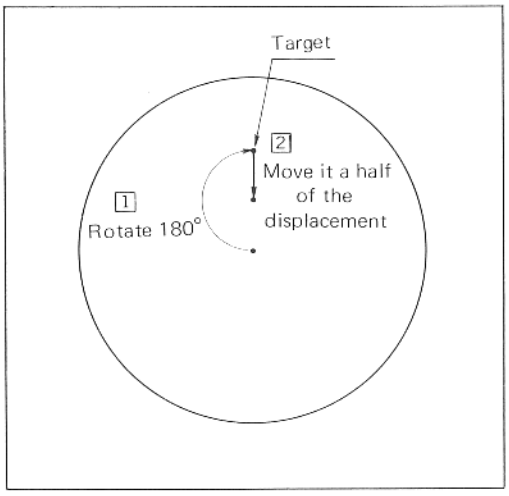


Fig. 18

4) Change over to the 40X objective, and follow the same procedure as with the 10X objective to check centration.
5) Tighten the stage rotation clamp screw.
6) Remove centering tools by pulling straight out. Do not twist to remove, as this will decenter the stage. Keep centering tools for future use.

IV. MICROSCOPY

1. Operating procedure

1) Turn the power switch located on left side of base to the ON position and adjust the voltage to 7 on the voltmeter.
2) Remove the lamp centering target and insert the filters to be used. For general microscopy, push in the blue filter button (NCB10) and the dark gray filter button (ND32) simultaneously.
3) Place the specimen on the stage and swing the 10X objective into position. Focus on specimen.
4) Adjust the interpupillary distance and diopter correction ring. (Refer to p. 11)
5) Make certain of correct illumination. (Refer to p. 10)
6) Carry out the centering procedure for the condenser. (Refer to p. 11)
7) Swing in the objective to that to be used and refocus on specimen.
8) Adjust the condenser. (Refer to Table 1)

Table 1. Use of Condensers

Type of condenser / Working distance / Objective	Swing-out condenser N.A. = 0.9 Dry system	Achromatic/aplanatic condenser N.A. = 1.4 Oil immersion	Function of diaphragms	
			For stopping down aperture (At the condenser)	For limiting illuminated field (On the microscope base)
	2.7 mm	1.7 mm		
2X 4X	Top lens swung out		*	Use aperture diaphragm
10X 20X 40X 100X	Top lens swung in	Usable	Use apeture diaphragm	Use field diaphragm

* To be fully opened

Note : The above working distance includes a glass slide thickness of 1.2mm.

9) Brightness is adjusted by selecting ND filters or by changing the lamp voltage.
10) Adjust the condenser aperture diaphragm and the field diaphragm. (Refer to p. 13)

2. Manipulation of each element

1) Selection of filters.

The black push button on the right side of the microscope base removes all filters from the optical path.

Depress one or more of the filter buttons simultaneously and the respective filters will be brought into the optical path.

To use another filter additionally, push the respective button, while keeping depressed the other buttons previously selected.

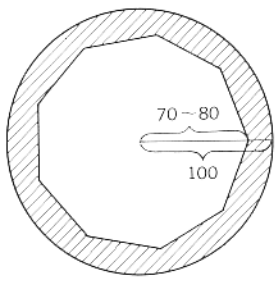
The filters, built-in the microscope base, are as shown below.

Table 2. Filters in the Base

Color of push button	Type of filter	Use
Light gray	ND2 (T = 50%)	For general microscopy and brightness adjustment in photomicrography
Gray	ND8 (T = 12.5%)	
Dark gray	ND32 (T = 3%)	
Blue	NCB 10 (Color temperature changing filter)	For general microscopy and color photomicrography
Green	Green	For phase-contrast observation and contrast adjustment in monochrome photomicrography

2) Adjustment of condenser aperture diaphragm

The condenser aperture diaphragm is provided for adjusting the numerical aperture (N.A.) of the illuminating system of microscope. It is important because it determines the resolution, contrast and depth of focus. In general, 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. 19)



Size of the condenser aperture diaphragm

Fig. 19

To adjust the size of the condenser aperture diaphragm, turn the diaphragm ring referring to the N.A. scale. Or, after removing the eyepiece from the microscope tube, make such adjustment, observing the image of the diaphragm which is visible on the exit of pupil of the objective is a bright circle inside the microscope tube. A phase telescope is recommended for greater accuracy.

Stopping down the aperture diaphragm too

far will deteriorate the image quality of microscope due to diffraction of light. Therefore, it is not recommended to stop down the aperture to a size smaller than 60% of the N.A. of the objective in use except when observing almost transparent specimens.

3) Adjustment of field diaphragm

The field diaphragm is used for determining the illuminated area on the specimen surface in relation to the field of view of the microscope. If the former be larger than the latter, extraneous light will enter the field of view, causing flare in the image and lowering the contrast.

Therefore, especially in photomicrography, the proper adjustment of the field diaphragm is very important. Generally, good results will be achieved when the diaphragm is stopped down to such an extent that the diameter of illuminated area is slightly larger than the diagonal of film format.

4) Focusing

The relation between the direction of rotation of the focus knobs and that of vertical movement of the stage is as indicated in Fig. 20.

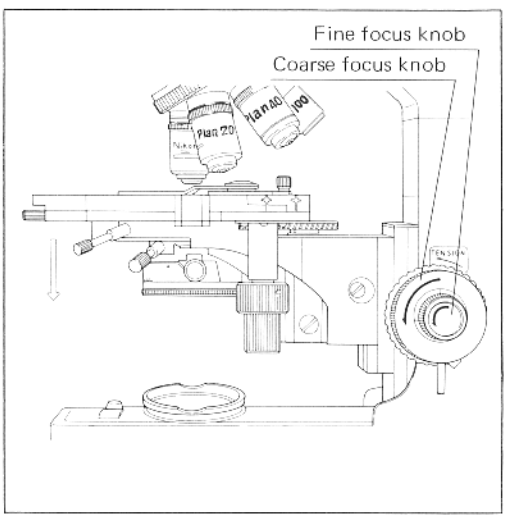


Fig. 20

The tightness of rotation of the coarse focus knob is adjustable. For increased tightness, rotate the tension adjuster surrounding the coarse focus knob in the direction of the arrow, labeled "Tension".

5) Preset focus lever.

After focusing, push the preset focus lever toward SET STOP. This prevents the stage from rising above the focused position with the coarse focus. This lever is especially useful with an oil immersion objective to prevent the specimen from making contact with the objective.

6) Replacing the revolving nosepiece

The revolving nosepiece is detachable. Release the clamp screw. Push the nosepiece against the clamp screw, tilt the side opposite the clamp screw, as shown in Fig. 21, to remove the nosepiece.

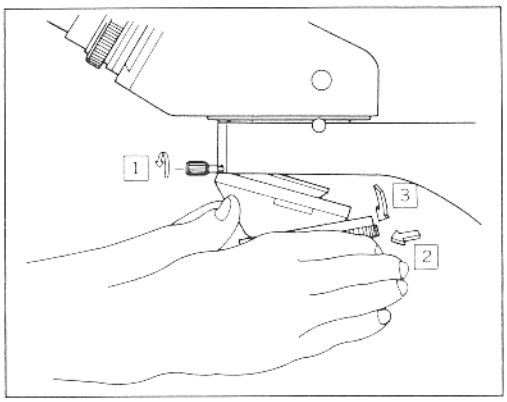


Fig. 21

For attaching, reverse the above procedure, as shown in Fig. 11. Therefore, revolve the nosepiece as a whole, until the pin settles in the groove. In this position, tighten the clamp screw.

V. OPTICAL SYSTEM

The CF objectives and CF eyepieces adopted in the Nikon V-series microscopes are designed on the basis of a new Nikon-developed concept "Chromatic Aberration Free". With the Nikon CF optical system the chromatic difference of magnification in the objective and eyepiece is individually corrected. This is unlike conventional microscopes where the corrections of such aberration has been, for the most part, compensated for in the objectives and eyepiece as a pair. As a result the Nikon V-series has no orange colored fringe in the eyepiece. In cooperation with the other optimum aberration corrections such as the Nikon Integrated Coating, a uniformly sharp image, much superior in resolution, contrast and color rendition is achieved over 100% of the effective, even, super-wide field of view, for observation as well as color photomicrography.

1. Objectives

In every case use the CF objectives in combination with the CF eyepieces. Mechanical tube length of 160mm and parfocal distance of 45 mm, (This is longer than the 33.6mm of earlier microscopes). To afford the optical designer a wide freedom for correcting aberrations.

1) Types of objectives

(1) Plan-achromat (Plan)
These objectives offer a flat image place of high definition over the field of view from center to periphery, and are most suitable for photomicrography as well as for observation.

(2) Plan-apochromat (Plan Apo)
Being of high numerical apertures and color aberration corrected to the highest degree over the whole range of visual light by using of fluorite and new type optical glasses, these objectives provide superior resolution, color rendition and image flatness, and are suitable for resolving the finest structures and for photomicrography. They also permit ultra-wide field of view observation.

(3) Plan fluorite (Plan Fℓ)
The plan semiapochromats with better color correction than the plan achromats, afford superior resolution and image contrast.

2) Use of the objective

(1) "Oil" immersion objectives
The objectives discriminated by the engraving "Oil" are to be immersed in oil between the specimen and front of the objective.
To realize the full advantages of such fluid-immersion objectives with an N.A. of 1.0 or higher, it is necessary to use the achromatic/aplanatic condenser and to fill the space between the top lens of the condenser and the bottom surface of the glass slide with immersion oil.
To see if air bubbles are present in the immersion oil, which deteriorate the image quality, pull out the eyepiece from the microscope tube to examine the objective exit pupil inside the tube.
To remove air bubbles, revolve the nosepiece slightly to and fro several times, apply additional oil, or replace the oil.
Be careful not to rotate the nosepiece too far as to soil the ends of the other objectives with oil.
To clean off the oil, pass lens tissue or soft cloth moistened with xylene lightly two or three times over the lens. It is essential at this time to avoid touching the lens with the part of tissue or cloth once used.
Any remnants of oil left on the lens deteriorate the image quality.

(2) Coverglass
With the objectives marked "160/0.17", use a coverglass of 0.17mm in thickness (No. 1½). For the objectives whose N.A. is 0.75 or higher, a coverglass of other thickness than 0.17mm will deteriorate the image definition and contrast.
The indication 160/– on the objective means that no matter whether a coverglass is used or not, no decrease of image definition or of contrast will result.

(3) Objectives with compensation ring
When a high power, dry objective of large N.A. is adopted in combination with a coverglass of thickness other than 0.17mm, which will cause sharp reduction of image definition and contrast, it is necessary to use an objective

incorporating a compensation ring as below:
First, observe with the compensation ring set to 0.17 and then rotating the ring, focus the image with the fine focus knob, until an image of the highest sharpness and contrast is obtained.

(4) No-coverglass objectives (NCG)
Objectives with the indication NCG are suited for observing specimens such as smears without coverglass.

(5) Objectives with aperture diaphragm adjustment
To be used for darkfield microscopy, the objective is equipped with an iris diaphragm which cuts off direct light entering the objective thus removing any glare when stopped down to nearly the smallest size.

2. Eyepieces

To take full advantage of the CF eyepieces, use in combination with the CF objectives. The indication "CF" should serve to prevent their use with other type objectives.

1) CFW eyepieces (CFW)
Being of wide field and high eyepoint type, the CFW eyepieces are only used for observation. They are equipped with an extensible eyeguard.

2) CFUW eyepiece (CFUW)
Featuring extra-wide field of view and high eyepoint, this eyepiece is designed exclusively for observation. It enables observation over a field of view twice as large as that of the ordinary type eyepieces in combination with the Ultra-wide field body tube. Each eyepiece is provided with an adjustable diopter ring.

3) CF Photo eyepieces (CF Photo)
Exclusively designed for photomicrography. Do not use them for observation. Every eyepiece is liable to gather dirt and dust, which not only appear as shadows but also impair image quality and contrast. Keep the eyepieces clean at all times.

3. Condensers

1) Swing-out condenser
It is used in combination with objectives from 2X to 100X, and provided with a swing-out top lens which is to be swung out when using the 2X or 4X objective.
The scale of its adjustable aperture is graduated in N.A. ratings.

2) Achromatic/aplanatic condenser
N.A. = 1.4. The spherical, coma and chromatic aberrations being ideally corrected, this large aperture condenser is used with 10X – 100X objectives. The standard thickness of glass slide should be 1.2mm.
Apply oil between the condenser and glass slide. It is recommended that this condenser be employed especially in combination with the Plan-Apochromatic objectives.

3) Darkfield condenser
N.A. is adjustable 1.43 – 1.20. A fluid (oil or glycerine) immersion system for darkfield use. The 10X – 100X objectives are applicable.

4. Illumination system (Refer to Fig. 22)

The optical system for illumination in the BIOPHOT microscopes is constructed to fulfill the Koehler illumination requirements perfectly, and offers a bright, uniform field without any change-over manipulation.
As a standard light source, use the halogen lamp 12V 100W. Five most commonly used filters are built in the microscope base. Usually, place the built-in diffuser in the optical path, but swing aside when the lamp is to be centered.
The VBD-type BIOPHOT microscope base has additional optical systems built in to the arm and base to enable using a second light source, such as a high-pressure mercury lamp. Furthermore, the stand of the microscope incorporates a detachable diffuser, permitting general observation with the high-intensity light source. There is a provision also for replacing the diffuser with excitation filters for diascopic fluorescence microscopy.

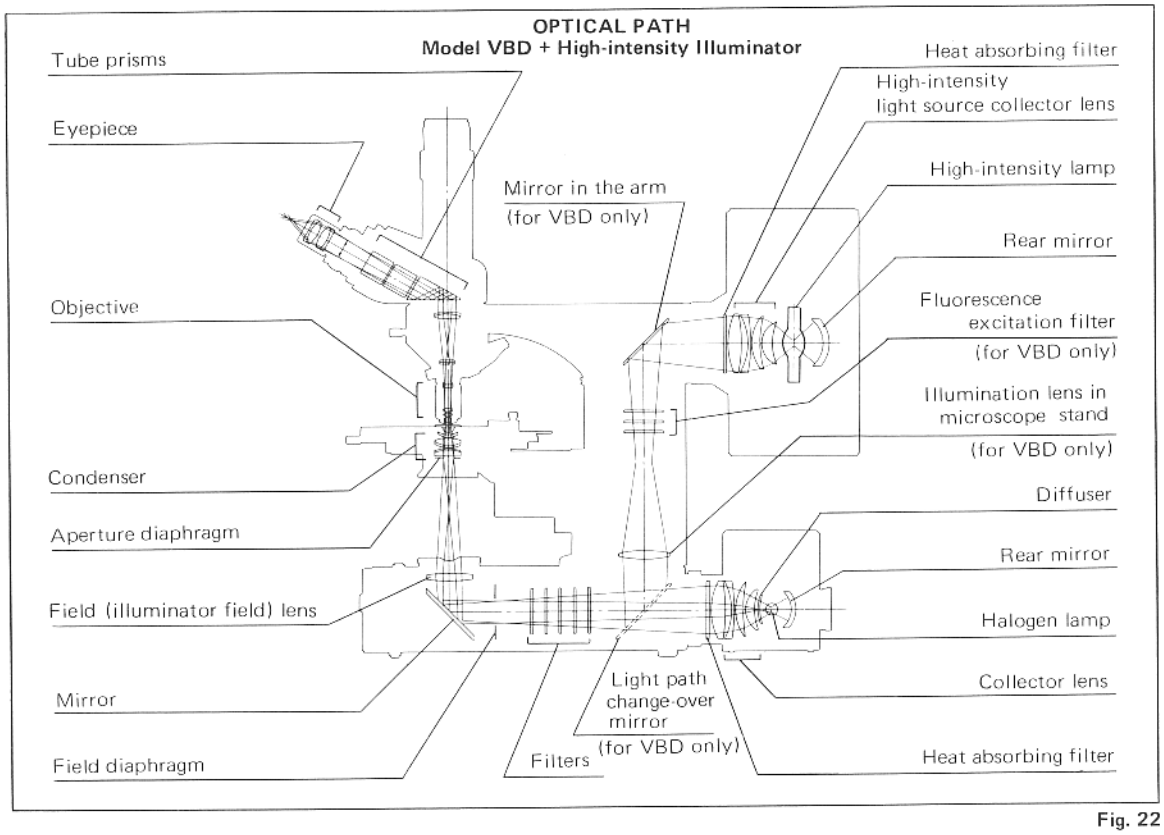

OPTICAL PATH
Model VBD + High-intensity Illuminator
Tube prisms
Eyepiece
Objective
Condenser
Aperture diaphragm
Field (illuminator field) lens
Mirror
Field diaphragm
Mirror in the arm
(for VBD only)
Filters
Light path
change-over
mirror
(for VBD only)
Heat absorbing filter
High-intensity
light source collector lens
High-intensity lamp
Rear mirror
Fluorescence
excitation filter
(for VBD only)
Illumination lens in
microscope stand
(for VBD only)
Diffuser
Rear mirror
Halogen lamp
Collector lens
Heat absorbing filter

Fig. 22

VI. PHOTOMICROGRAPHY

The BIOPHOT microscopes are designed with particular care to assure the finest photomicrographs. Although various types of Nikon photomicrographic attachments (Microflex) are mountable, it is especially recommended that the Microflex Model HFM be used.

1. Combination of CF objectives and CF eyepieces

The combined use of the CF objectives and CF eyepieces is essential. For the same total magnification, select a combination of the highest possible objective power and lowest possible eyepiece power to achieve the utmost image definition and contrast.

2. Checking the illumination

Unevenness in the illumination will show up more conspicuously in photomicrography than in observation. Consequently, before taking a photograph, recheck the positioning and centering of the lamp and the correct adjustment of the condenser.

3. Voltage for the lamp

1) For color photomicrography
The color temperature of the light source varies with the lamp voltage. In the BIOPHOT, the built-in filters are so selected as to enable one to take color correct pictures with the lamp voltage set to "9" in almost all cases when a daylight film is used.

2) For monochrome photomicrography
In this case, make exposures with the voltage set at "6" or higher.

4. Selection of filters

For photomicrography, the selection of filters is important. Understand the characteristics of each filter thoroughly.

1) For color photomicrography
When the color film is daylight type, use the filter NCB10 (blue push button) in the microscope base. The NCB10 filter being a combination of color temperature conversion and color compensation types is most suitable for a standard film. Depending upon the make of the film used different color renditions may result. It is recommended that in addition to the NCB10 filter a color compensation filter (CC filter), available from the film manufacturer, be used.
For the characteristics of the various filters, refer to the data given on p. 25.

2) For monochrome photography
In this case, the NCB10 filter (with blue push button) built in the microscope base is to be brought out of the optical path. If the highest possible contrast is needed, insert the green filter (green push button) into the optical path.

5. Shutter speed

Desirable shutter speeds for least vibration are 1/4 ~ 1/15 sec. (Adjustment of the image brightness should be made by means of the ND filters built-in the microscope base).

6. Manipulation of field and aperture diaphragms

In photomicrography, the adjustment of the field diaphragm is important for the purpose of limiting extraneous light which causes flare in the microscope image.
Stop down the diaphragm so as to get an illuminated area slightly larger than that of the picture field. By adjusting the aperture diaphragm, a change of depth of focus, contrast and resolution of image is attainable.
Select a size suited to the purpose.
Generally speaking, the aperture diaphragm is properly stopped down to 70 ~ 80% of the aperture of the objective being used.

7. Focusing

Focusing is to be accomplished by means of the ocular finder on the photomicrographic attachment.
Use the ocular finder to obtain sharp focus on film plane.
For critical sharpness focus your eye first on the reticle using the diopter ring on the ocular finder then focus on the specimen.
For focusing, when using a low power objective, use the focusing telescope.

8. Vibration-free operation and photo support bracket

Set the microscope on a vibration-resistant, rigid desk or a bench with a vibration-proof device. Also use the photo support bracket.

9. Others

For the use of other photomicrographic attachments refer to the pertinent instruction manuals.

VII. USE OF THE ACCESSORIES

1. Ultra-Wide Field Eyepiece Tube

1) Nomenclature (Refer to Fig. 23)

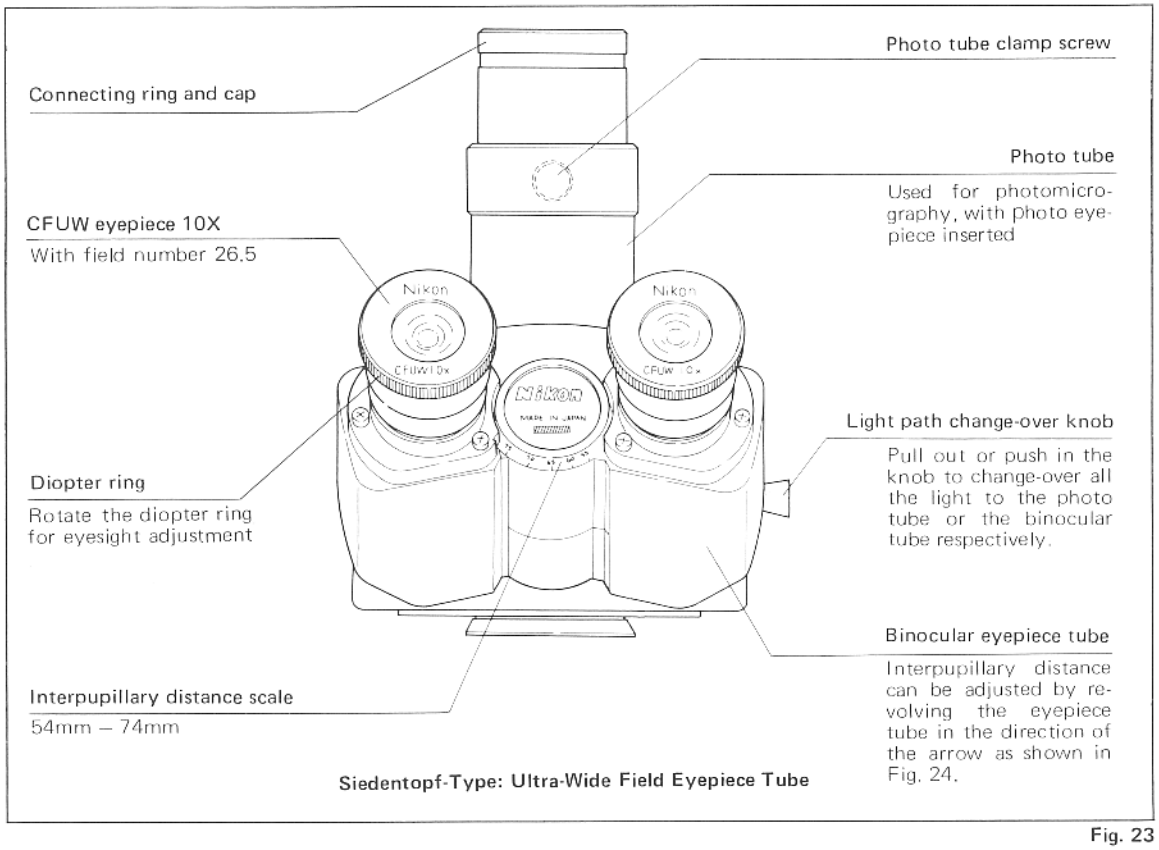


Fig. 23

2) Assembly

(1) Attaching the UW eyepiece tube
Loosen the clamp screw sufficiently and tilt the UW tube, and press it toward the clamp screw, place it in the horizontal position. Tighten the clamp screw.

(2) Attaching the CFUW eyepiece
Bring the notch on the eyepiece sleeve into coincidence with the positioning pin on the CFUW eyepiece. Attach the eyepiece.

(3) Objective
CF Plan-achromat or CF Plan-apochromat objectives, 4X – 100X are used.

(4) Condenser
Use the swing-out condenser.
(Achromatic/aplanatic condenser is usable with 20X – 100X objectives.)

3) Microscopy
The use of CFUW eyepieces being almost the same as that of the regular CF eyepieces (refer to p. 13), only the differences will be described below:

(1) Adjustment of interpupillary distance
Hold the binocular eyepiece tube with both hands as shown in Fig. 24. Looking into the eyepieces, adjust the distance to unite right- and lefthand fields of view.

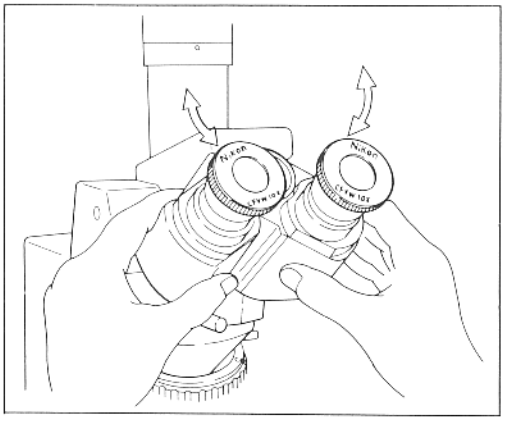


Fig. 24

(2) Eyesight adjustment

Change-over to 40X objective. Looking into the righthand eyepiece, manipulating the coarse and fine focus knobs to bring the specimen image into sharp focus. Thereafter, change-over to the 4X objective. Rotate the diopter ring on the righthand and then that on the lefthand eyepiece for sharp focus (Refer to Fig. 25)

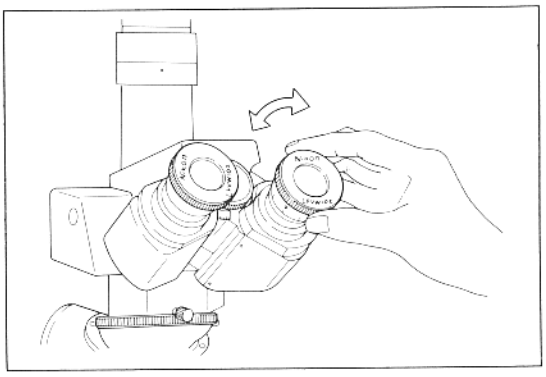


Fig. 25

2. Phase-contrast Equipment

1) Nomenclature (Refer to Fig. 26)

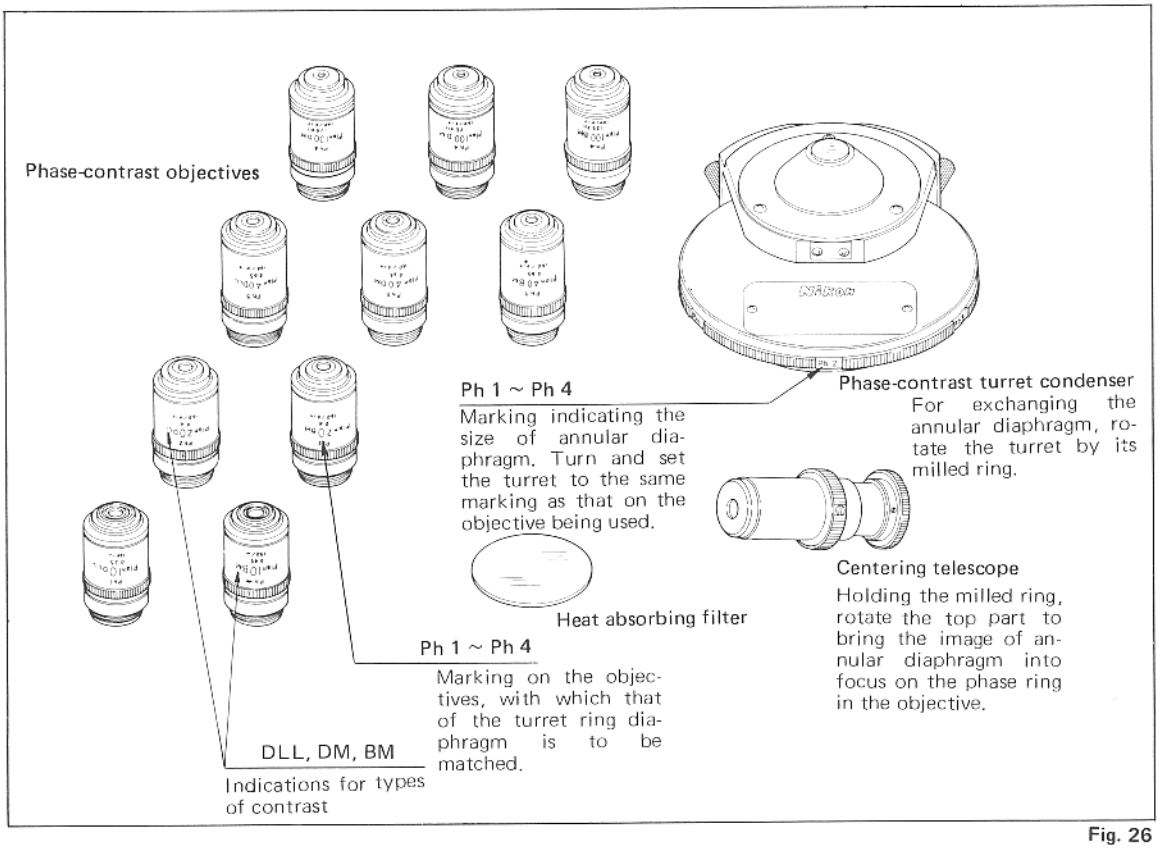


Fig. 26

2) Assembly

(1) Attaching the objectives

1. Remove the revolving nosepiece and screw in the phase-contrast objectives.
2. The objectives are to be arranged on the nosepiece in such an order that, when viewed from above, the magnifying power increases as the nosepiece is revolved clockwise, as shown in Fig. 27.

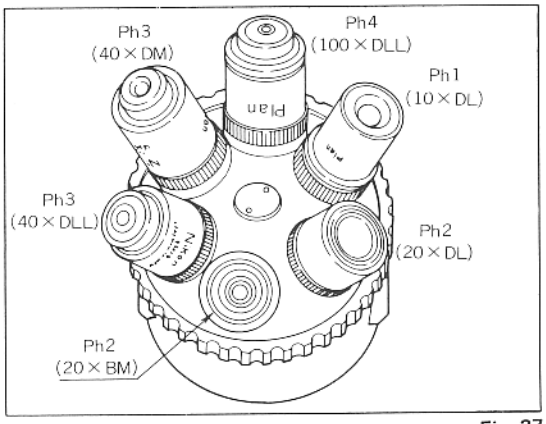


Fig. 27

3. Release the nosepiece clamp screw. Tilt the nosepiece with the objectives attached, and press the nosepiece against the clamp screw, as shown in Fig. 28. Seat it in horizontal position.

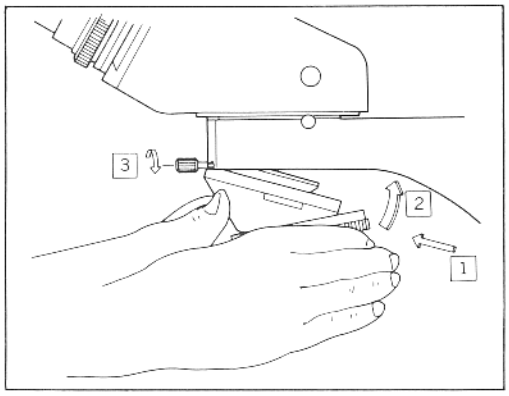


Fig. 28

4. Revolve the nosepiece until the pin settles in the groove, lock the clamp screw in this position.

(2) Attaching the phase turret condenser

1. Lower the condenser mount and release the clamp screw on the condenser carrier and remove the carrier.
2. Slide the phase-contrast turret condenser over the attaching dovetail. Mount it in position as shown in Fig. 29. Tighten the clamp screw.

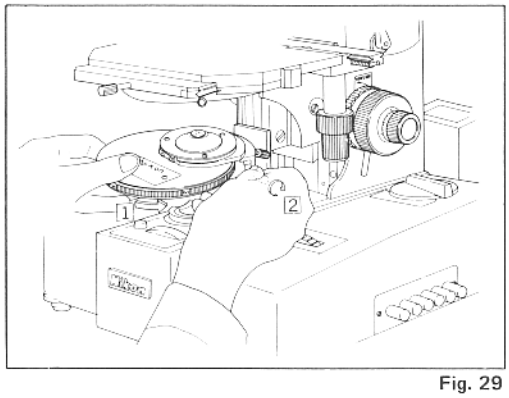


Fig. 29

3. Raise the turret condenser to its highest position by use of the condenser vertical motion knob.

3) Phase-contrast microscopy

The procedure is almost the same as that for the ordinary bright-field microscopy (refer to p. 13) with the exceptions noted below:

(1) Use of filters

1. For observation and photomicrography with monochromatic light, use the green filter (green push button) in the microscope base.
2. For microscopy with white light, use the NCB10 filter (with blue push button).
3. To protect a specimen from deterioration or a living specimen from dying under the heat of the illuminating light, place a heat absorbing filter (45mm in outer diameter) over the field lens in the microscope base.

(2) Centering the condenser

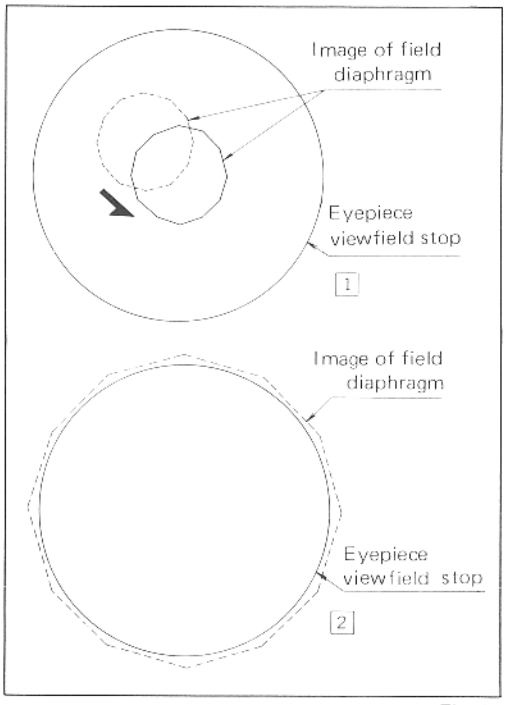


Fig. 30

1. Set the condenser turret to 0 position and revolve the objective nosepiece to the 10X objective. Focus on the specimen.
2. Rotate the field diaphragm ring on the microscope base. Stop down the diaphragm to its smallest size. Rotate the condenser vertical motion knob to bring the image of the field diaphragm into focus on the specimen surface.
3. If the field diaphragm image is decentered in relation to the eyepiece viewfield, adjust by means of the condenser centering screws.
4. Change-over to the 40X objective.

Adjust the size of the field diaphragm image to almost the same as that of the eyepiece viewfield. If it is decentered again, adjust by means of the condenser centering screws, as shown in Fig. 30.

(3) Centering the annular diaphragm

1. Turn the turret to the same Ph marking as that of the objective being used.
2. Remove one eyepiece and in its place insert the centering telescope. Holding the milled part of the telescope, rotate its eyepiece until the image of the phase-contrast ring in the objective is brought into focus. The image of the condenser annular ring will also be visible.
3. If the annular ring image is decentered with reference to the phase-contrast ring in the objective adjust by pushing the annular ring on the turret with both fingers, as shown in Fig. 31.
4. For the other objectives, follow the same centering procedure as the above.

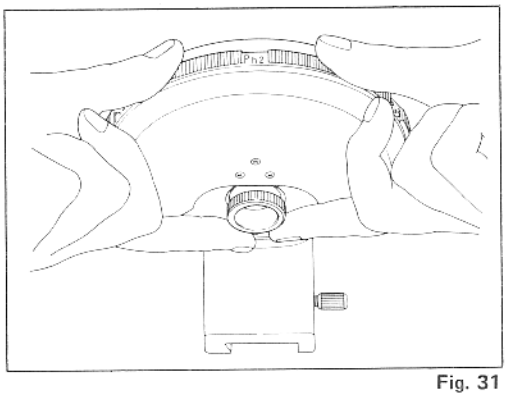


Fig. 31

3. Simplified Polarization Equipment

1) Nomenclature

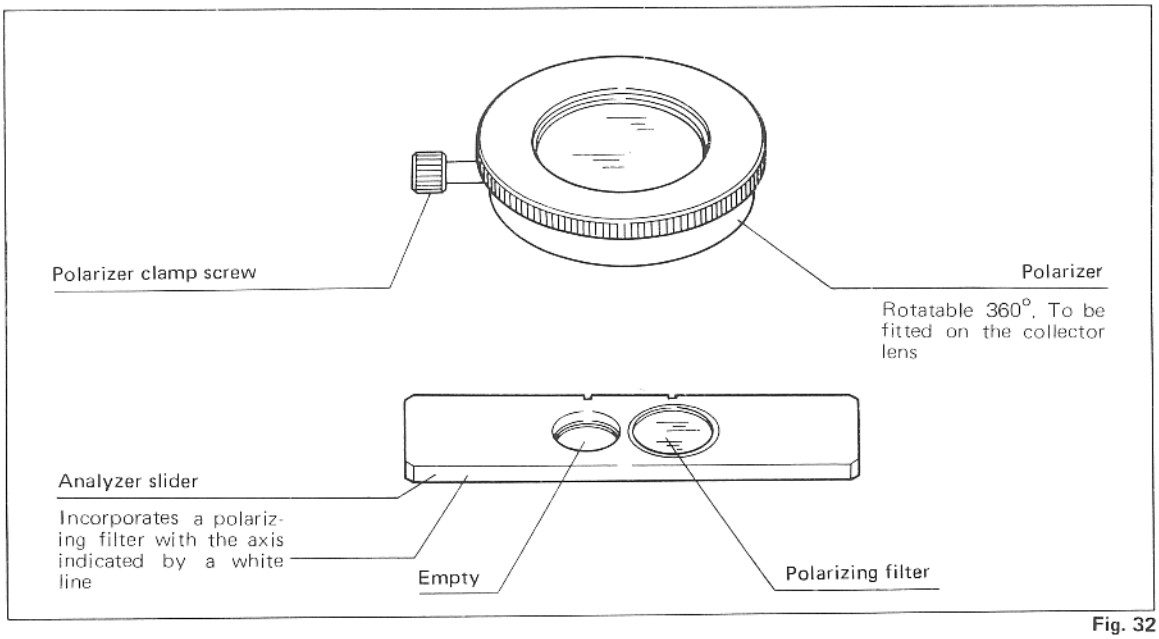


Fig. 32

2) Attaching the analyzer slider

(1) Remove the filter slider by pushing out of the revolving nosepiece, and in place insert the analyzer slider in such a position that the groove on the slider faces toward the back as shown in Fig. 33.

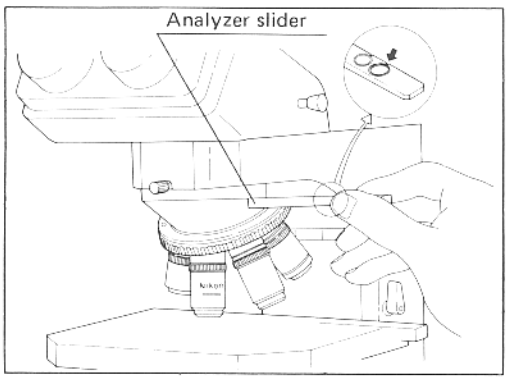


Fig. 33

(2) Condenser

The swing-out condenser (Refer to p. 16) is recommended.

(3) Objectives

Use the regular CF objectives.

(4) Attaching the polarizer

Fit the polarizer to the top of the field lens, as shown in Fig. 34, and fasten it in position with the lock screw.

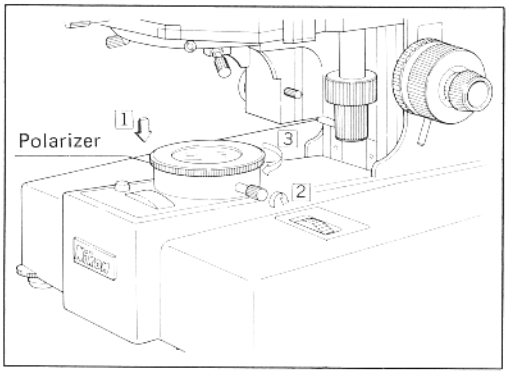


Fig. 34

3) Polarized light microscopy

1. Turn ON the power switch.
   Set the lamp voltage at 7 – 8 by means of the brightness control knob.
2. Remove the lamp target and place the NCB10 filter (blue push button) into the optical path.
3. Place the specimen on the stage and focus using the 10X objective.
4. Adjust the interpupillary distance and set diopter adjust. (Refer to p. 11).
5. Place the swing-out condenser in the optical path. (If using the 4X objective swing out the top lens.)
6. Center of the condenser (Refer to p. 11).
7. Rotate the polarizer until the darkest field of view is obtained.
8. Adjust the lamp voltage to 10 – 11 by means of the brightness control knob.
9. Change-over to the objective to be used and sharpen the focus on the specimen.
10. Adjust the aperture diaphragm and field diaphragm. (Refer to p. 13)

4. Projection Screen

1) Nomenclature

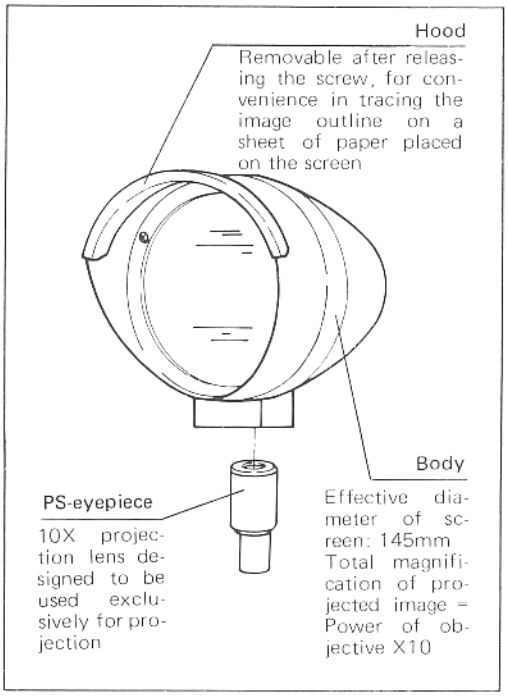


Fig. 35

2) Assembly

(1) Attaching the PS eyepiece

Insert the eyepiece into the photo tube sleeve of the trinocular eyepiece tube.

(2) Attaching the screen body

Mount the screen body on the photo support bracket, or use the connection ring on the photo tube of the trinocular eyepiece tube.

3) Observation

(1) Use the NCB10 filter (blue push button) built-in the microscope base.

(2) Switch ON power. Set the lamp voltage to 10 – 11 by means of the brightness control dial.

(3) On the trinocular eyepiece tube pull the light path change-over knob until all light goes to the screen.

(4) The other manipulations are the same as for ordinary brightfield microscopy. (Refer to p. 17)

It is recommended that the screen and the surroundings be made as dark as possible.

Note: The screen surface being matte, be careful not to leave finger marks, grease or the like.

To clean the screen surface wipe it, using cotton cloth or lens tissue moistened with xylene, alcohol or ether.

SOME DATA ON COLOR PHOTOMICROGRAPHY USING BIOPHOT MICROSCOPES

We have designed the BIOPHOT microscopes to attain correct color rendition without the use of any additional filters with common type films. However, depending upon the type of film, emulsion number, conditions of development, as well as staining of the specimen, delicate changes of color reproducibility may unavoidably occur. Therefore, it is sometimes necessary to use additional color compensating filters.*1

In the following table are given some of the data we have obtained by our tests which have been made for typical color films under the standard photomicrographic conditions, for reference:

Name of film (ASA film speed)	Filter, built in (NCB10)	Lamp voltage	Additional filter (CC-filter)	Film speed (ASA) setting	Shutter *2 speed setting (Range) sec.	*3 Characteristics of film
Kodachrome 25 DAYLIGHT (25)	Used	9	Not used	25	1/15* (1/10~1/30)**	Good resolution, color balance and background tone
Kodachrome 64 DAYLIGHT (64)	Used	9	Not used	64	1/15* (1/10~1/30)**	Almost corresponding to Kodachrome 25, with a slight yellow tinge in the background
Ektachrome X DAYLIGHT (64)	Used	9	Not used	64	1/15* (1/4~1/30)**	Good balancing of colors and easily usable
Kodak PCF 2483 (16)	Not used	9	CC30G – CC40G	12~16	1/15* (1/10~1/30)**	Good resolution and contrast, but narrow latitude
Agfa CT18 (50)	Used	11	Not used	50	1/15* (1/4~1/30)**	Good resolution, color balance and background tone
Fujichrome R-100 (100)	Used	9	Not used	100	1/15* (1/2~1/30)**	Good balancing of colors and easily usable
Sakura R-100 (100)	Used	9	Not used	100	1/15* (1/2~1/30)**	Good contrast

* USED
** ACCEPTABLE RANGE

*1. CC-filters made by Kodak or Fuji Co.
To compensate the tinge as a whole:
Green, add CCM filter
Blue, add CCY
Pink, add CCG

*2. To compensate for the shutter speed, use a combination of ND filters built in the microscope base.

*3. Films are being improved constantly so please note that the above data should serve only as a rough guide.

(76. 8. c) H–1

Nikon

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