Nikon

Biological Microscope
OPTIPHOT

INSTRUCTIONS

NIPPON KOGAKU K.K.

CAUTIONS

1. When carrying the microscope, hold its arm with one hand, supporting the bottom of the microscope base with the other. The instrument weighs about 10.5 kg. **Do not have the lamp housing carry any load.**
2. Take care **that some parts of the lamp housing may take a considerable high temperature,** while the lamp is being lighted.
3. **Do not bring inflammable substances** such as gasoline, thinner, and alcohol **near to the lamp housing,** while the lamp is being lighted.
4. Never connect the lamp housing cord to the house current socket directly. **The rated power is 12V, 50W.**
5. **Do not use the 12V, 100W halogen lamp bulb.** If the lamp bulb of over-rated wattage is used, light adjusting circuit will damage.
6. Handle the microscope gently, taking care to **avoid sharp knocks.**
7. In such cases as of replacement, **do not touch the lamp bulb with bare hands,** immediately after putting out the lamp.
8. **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.
9. Do not leave **dust, dirt or finger marks** on the lens surfaces.
10. In every case, **make sure of the power source voltage** by means of the input voltage change-over switch on the bottom of the microscope base.
11. Before replacing the fuse, **disconnect the plug of the power source cord.**
12. **Never attempt to adjust the tightness** of the right- and lefthand focus knobs **by turning the one, while holding the other** in this model microscope, because of causing disorder.
13. **More rotation of the coarse focus knob after reaching the limit will cause the trouble.** Never rotate the knob beyond the rotation limit.

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 **absolute alcohol** (methanol or ethanol) be used.
   For cleaning the objectives and immersion oil use only xylene. For cleaning the surface of the entrance lens of the eyepiece tube and the prism surface of the Trinocular Eyepiece Tube "T" or the Ultra Wide Eyepiece Tube "UW", use absolute alcohol.
   Observe sufficient caution in handling alcohol and xylene.
2. **Avoid the use of any organic solvent** (for example, thinner, ether, alcohol, xylene etc.) 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 and fungus.**
   It is especially recommended that the objectives and eyepieces be kept in an airtight container containing desiccant.

CONTENTS

I . NOMENCLATURE

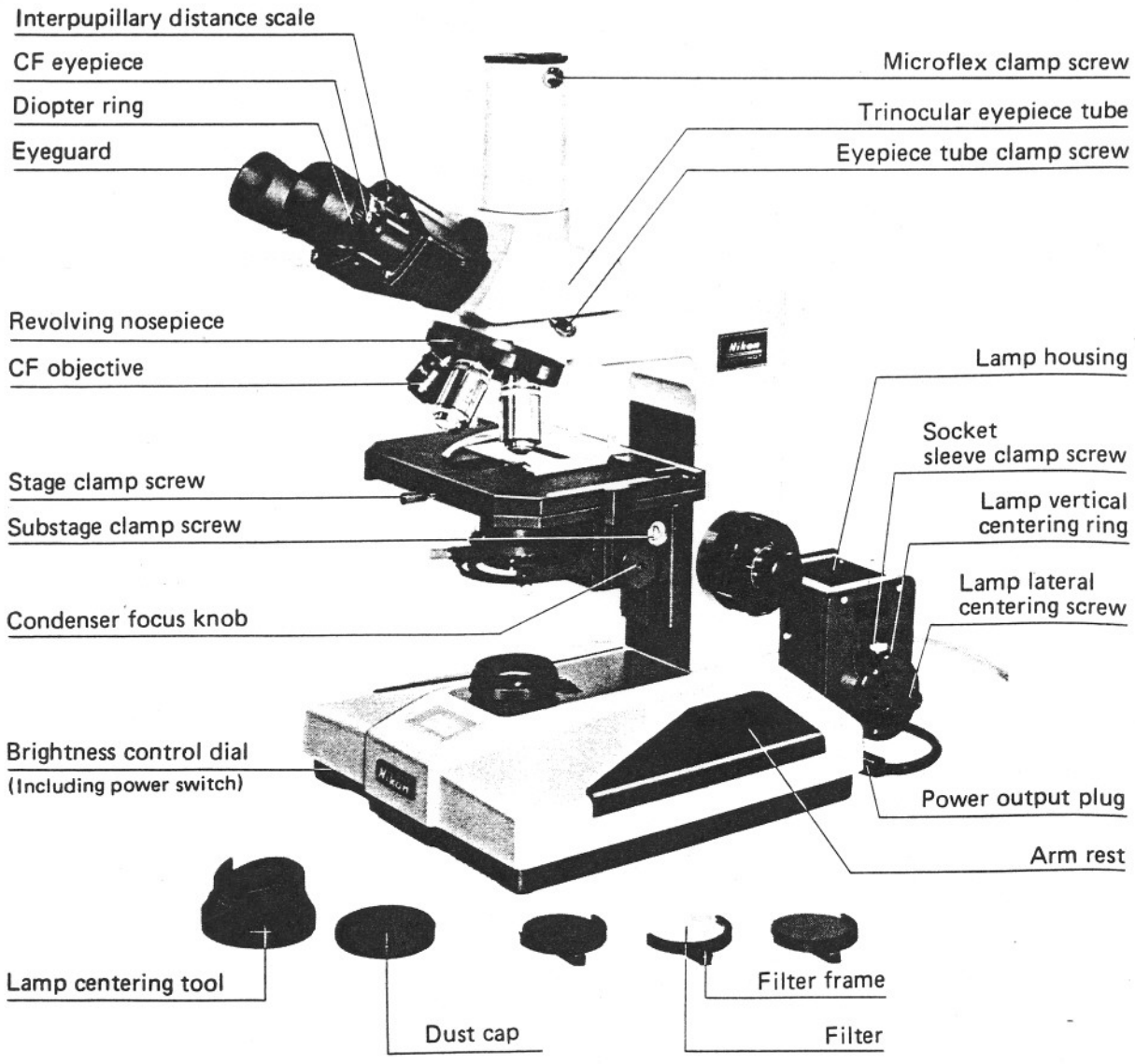


Fig. 1

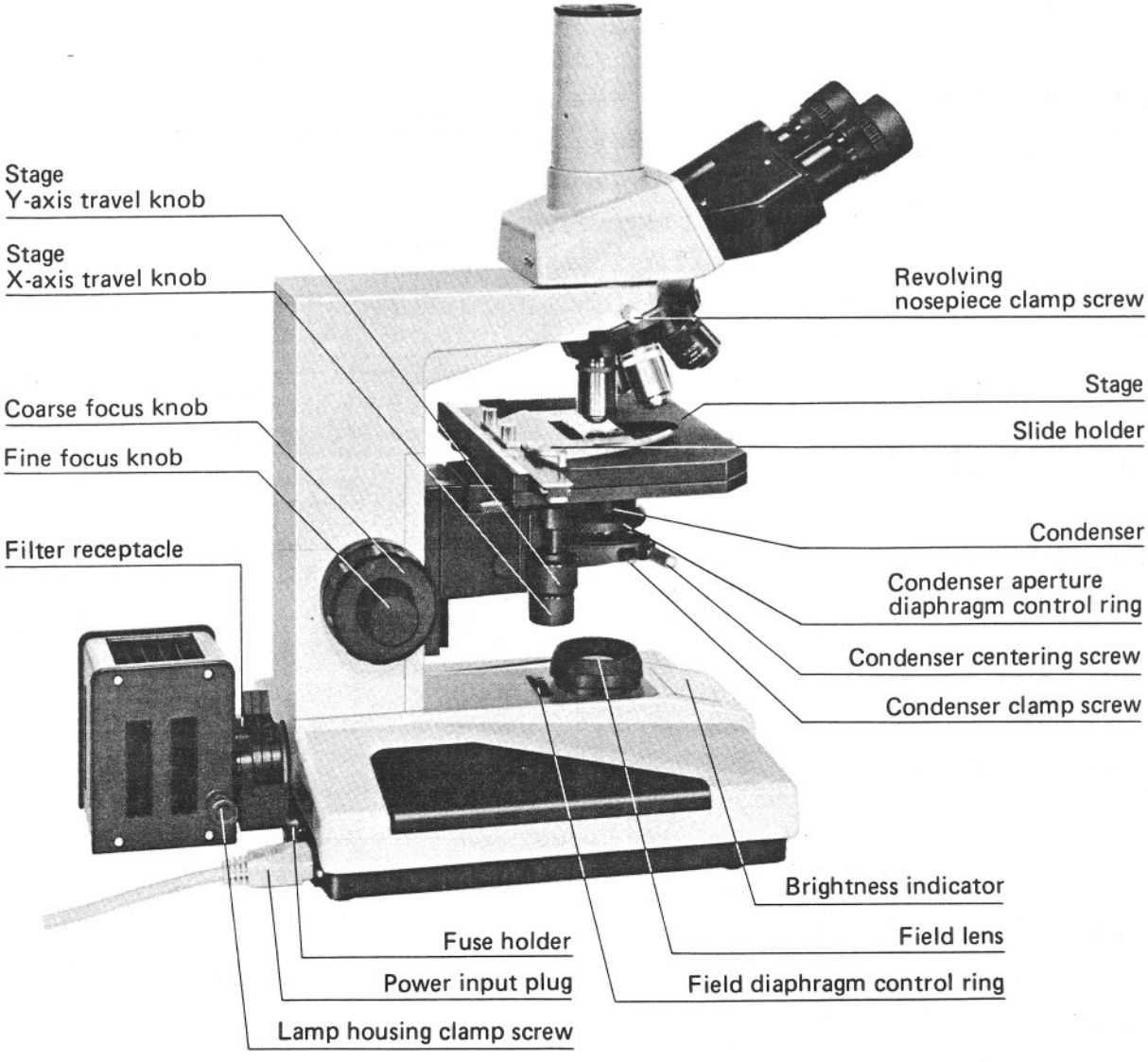

Stage
Y-axis travel knob
Stage
X-axis travel knob
Coarse focus knob
Fine focus knob
Filter receptacle
Revolving
nosepiece clamp screw
Stage
Slide holder
Condenser
Condenser aperture
diaphragm control ring
Condenser centering screw
Condenser clamp screw
Brightness indicator
Field lens
Field diaphragm control ring
Fuse holder
Power input plug
Lamp housing clamp screw

Fig. 2

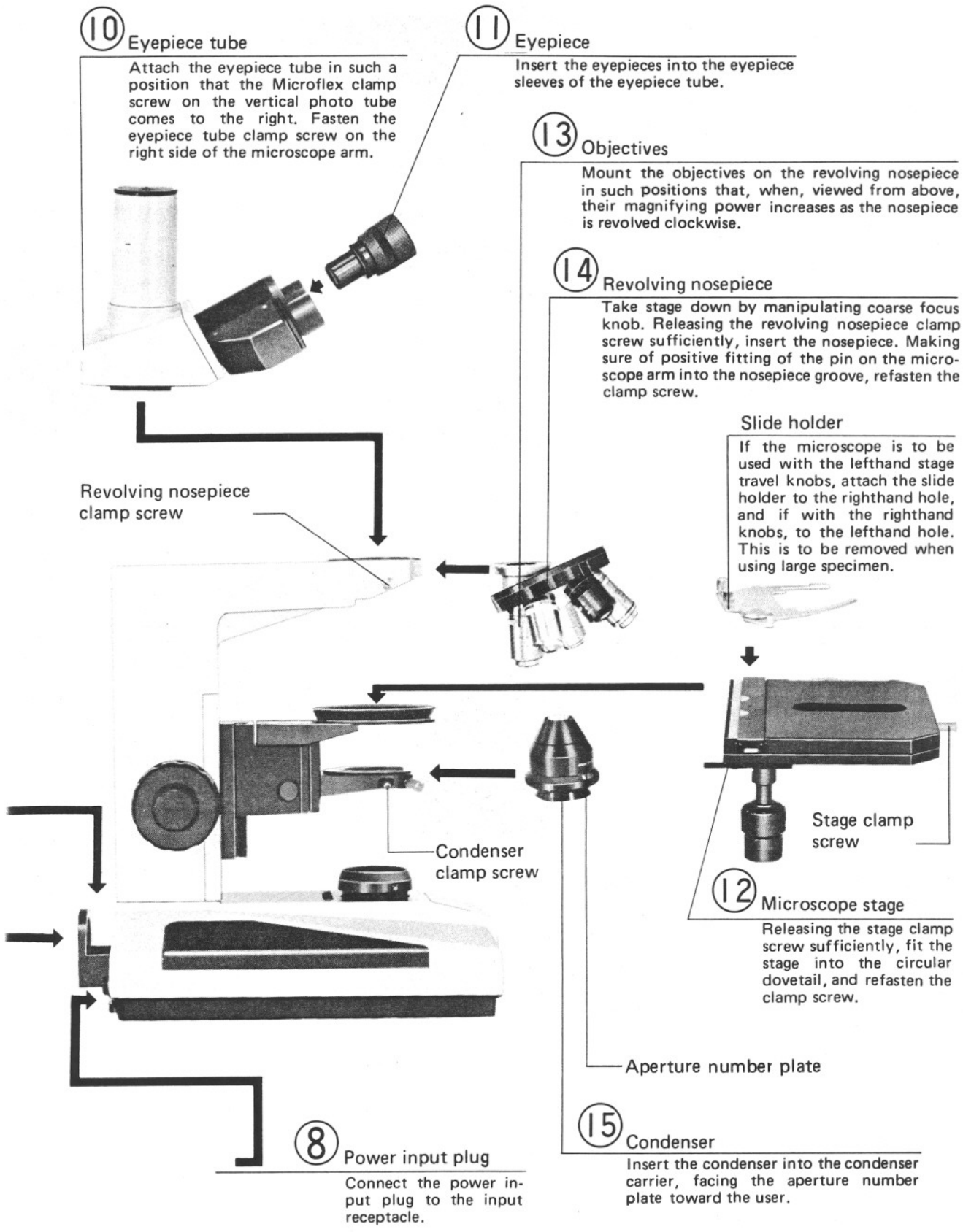

10 Eyepiece tube
Attach the eyepiece tube in such a position that the Microflex clamp screw on the vertical photo tube comes to the right. Fasten the eyepiece tube clamp screw on the right side of the microscope arm.
11 Eyepiece
Insert the eyepieces into the eyepiece sleeves of the eyepiece tube.
13 Objectives
Mount the objectives on the revolving nosepiece in such positions that, when, viewed from above, their magnifying power increases as the nosepiece is revolved clockwise.
14 Revolving nosepiece
Take stage down by manipulating coarse focus knob. Releasing the revolving nosepiece clamp screw sufficiently, insert the nosepiece. Making sure of positive fitting of the pin on the microscope arm into the nosepiece groove, refasten the clamp screw.
Slide holder
If the microscope is to be used with the lefthand stage travel knobs, attach the slide holder to the righthand hole, and if with the righthand knobs, to the lefthand hole. This is to be removed when using large specimen.
Revolving nosepiece clamp screw
Stage clamp screw
Condenser clamp screw
12 Microscope stage
Releasing the stage clamp screw sufficiently, fit the stage into the circular dovetail, and refasten the clamp screw.
Aperture number plate
8 Power input plug
Connect the power input plug to the input receptacle.
15 Condenser
Insert the condenser into the condenser carrier, facing the aperture number plate toward the user.

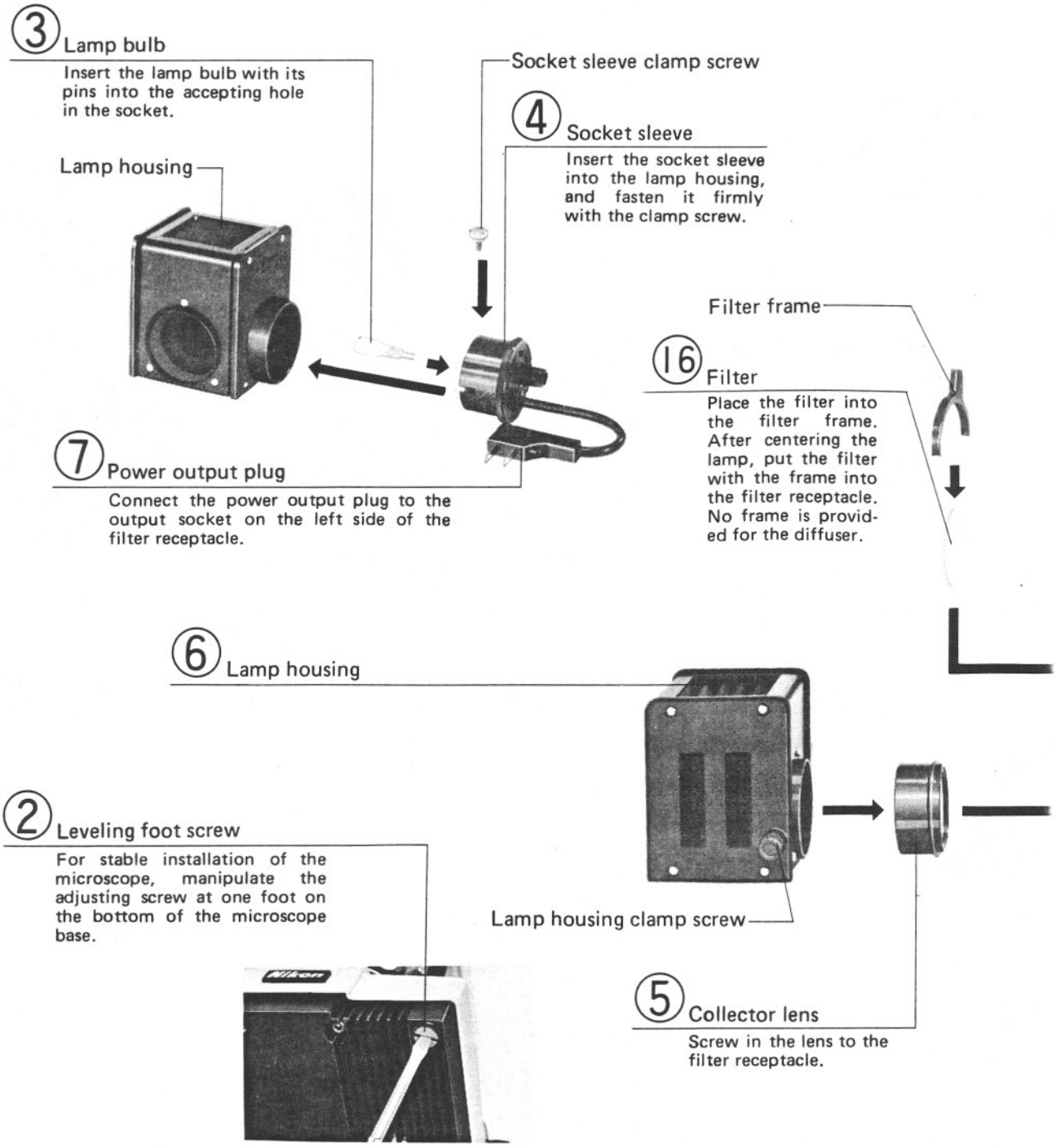
Fig. 3

II. ASSEMBLY

- To assemble the microscope, follow the procedure in the order given:

① Make sure of the power source voltage. (Refer to P. 8, III-2.-1))

⑨ Make adjustment of the lowest voltage. (Refer to P. 9, 3.)



III. PREPARATION

1. Mounting the Condenser

1) Lower the condenser carrier to its lowest limit.
2) Facing the aperture number plate toward the user, mount the condenser onto the carrier. Fasten up the condenser in this position by means of the clamp screw.
3) Raise the condenser carrier to its highest limit.

2. Centering the Lamp

1) Set the input voltage to the power source voltage by means of the change-over switch. (Fig. 4)

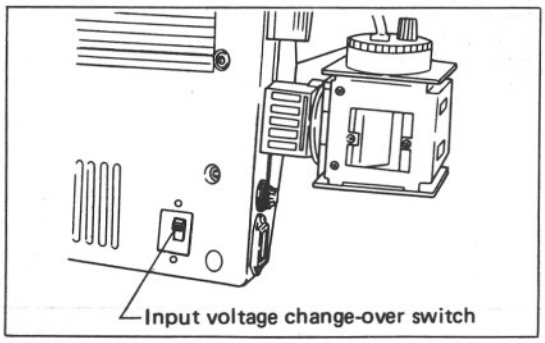


Fig. 4

2) Connect the power source cord to the socket.
3) Put the ND2 and ND16 filters into the filter receptacle.
4) Turn the brightness control dial to switch ON and adjust the voltage to 6 on the indicator.
5) Place the specimen on the stage, and **focus on the specimen using 10× objective.** In this case, **open the condenser aperture and field diaphragms** to the largest extent.
6) Roughly center the condenser lens using 10× objective, following the procedures given on P. 12 - 5).
7) Put the lamp centering tool on the field lens and onto the tool place a ND filter taken out of the filter receptacle. (Fig. 5)

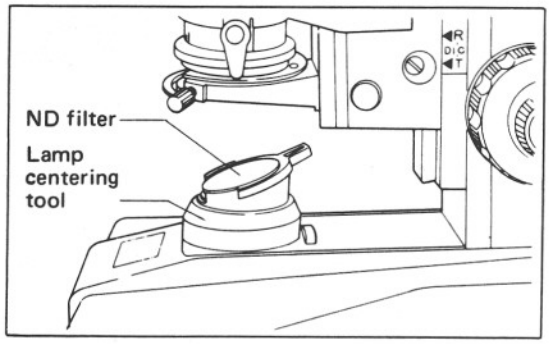


Fig. 5

8) Stop down the condenser aperture diaphragm, release the lamp housing clamp screw, and move the lamp housing back and forth (Fig. 6), until **a sharp image of the lamp filament appears on the aperture diaphragm surface,** which can be seen by the reflection from the ND filter.

Note: When using the achromat/aplanat condenser, a filament image (reddish violet) by the reflection from the lens surface is sometimes taken for a filament image (bluish white) appeared on the aperture diaphragm surface. In this case, stop down the field diaphragm, and the reddish violet-colored filament image will disappear and the bluish white-colored image is easy to see.

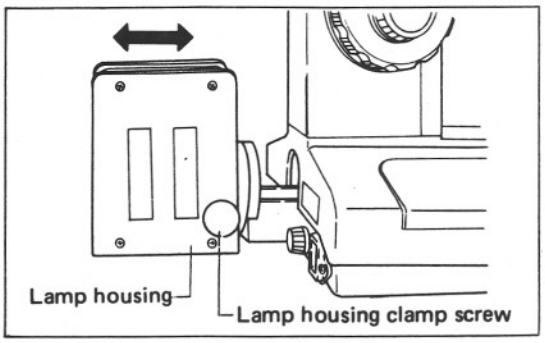


Fig. 6

9) Release the socket sleeve clamp screw (Fig. 7). Turning the lamp lateral centering screw and vertical centering ring, bring the filament image to the center, as shown in Fig. 8.

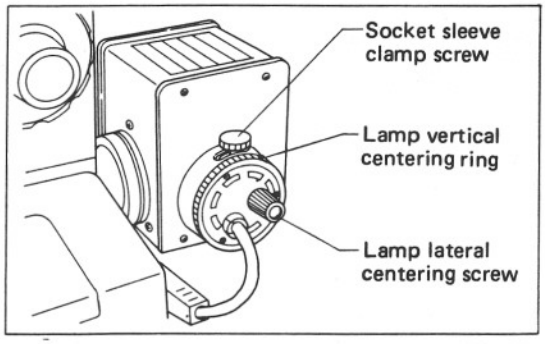


Fig. 7

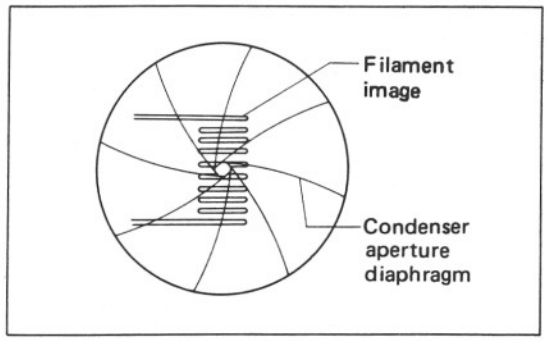


Fig. 8

10) As shown in Fig. 9, put the diffuser, with its matte surface faced toward the microscope stand, into the filter receptacle which is the closest to the microscope stand.

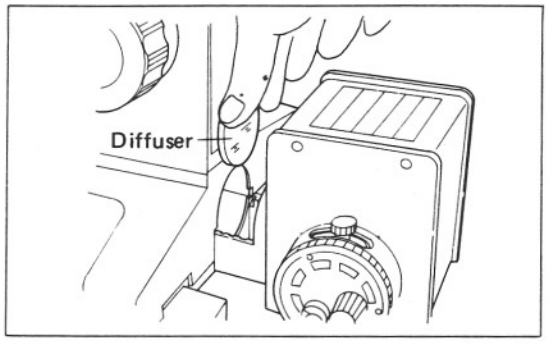


Fig. 9

The above centering procedure should be carried out, when replacing the lamp bulb.

3. Adjustment of the Lowest Power Source Voltage

If the illumination is found too bright or unstable, when the switch is turned ON, make adjustment of the lowest voltage in the following way:

1) Turn the brightness control dial to OFF.
2) Turn the lowest voltage adjusting screw on the bottom of the microscope base counter-clockwise to the limit, using a screw driver, as shown in Fig. 10.
3) Turn the brightness control dial to ON. At this time, the lamp voltage will be highest, immediately after lighting.
4) In this condition, gently turn the lowest voltage adjusting screw clockwise, to set the voltage to nearly 4 on the indicator.

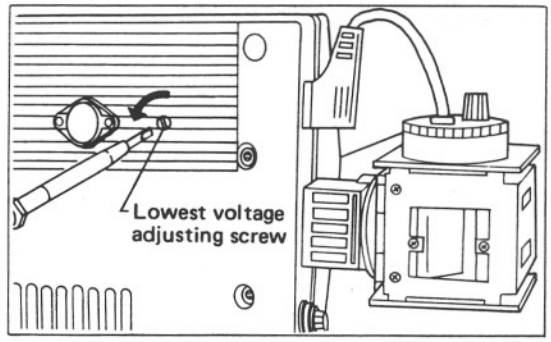


Fig. 10

IV. MICROSCOPY

1. Operating Procedure

1) Turn the brightness control dial (including power switch) to ON and adjust the voltage to 7 on the indicator.
2) Remove the dust cap and put the filters to be used. For general microscopy, put the NCB 10 filter, ND 2 filter, and ND 16 filter simultaneously.
3) Place the specimen on the stage and swing the 10× objective into position. Focus on specimen.
4) Adjust the interpupillary distance and diopter. (Refer to P. 11)
5) Make certain of correct illumination. (Refer to P. 8)
6) Carry out the centering procedure for the condenser. (Refer to P. 12)
7) Swing in the objective to be used and refocus on specimen.
8) Adjust the condenser. (Refer to Table 1)

Table 1. Use of Condensers

Type of condenser / Object distance / Objective	Swing-out Achromat condenser N.A. = 0.9 Dry system	Achromat/aplanat condenser N.A. = 1.35 Oil-immersion
	1.8mm	1.6mm
1×	Remove the condenser	Remove the condenser
※2× ※4×	Top lens swung out	
10× 20× 40× 100×	Top lens swung in	Usable

[NOTE]
- The above object distance (from the top of the condenser lens to the specimen surface) includes a glass slide thickness 1.2mm.
- ※ When using the Swing-out condenser with 2× or 4× objective, fully open its aperture diaphragm.
- For ultra-wide viewfield observation, the above table is applicable so far as an objective magnifying power of 2× or higher is used.
- For photomicrography using the 2× objective, preferably remove the condenser.
- For observation with the 1× objective, take off one ND filter, and in place insert the diffuser of 45mm in diameter (available as an accessory on order).

9) Brightness is adjusted by selecting ND filters or by changing the lamp voltage to 6 ~ 12.
10) Adjust the condenser aperture diaphragm and the field diaphragm. (Refer to P. 12, 13)

2. Manipulation of Each Element

1) Use of filters

Put the filter with the frame into the filter receptacle between the microscope base and the lamp housing. The accessory filters are as shown below:

Table 2. Use of Filters

Type of filter	Use
Diffuser (Without frame)	To be inserted in all cases except for lamp centering
ND 2 filter (T=50%) ND 16 filter (T=6.25%)	For general microscopy and brightness adjustment in photomicrography
NCB 10 filter (Color balancing filter)	For general microscopy and color photomicrography

2) Interpupillary distance adjustment

Place a specimen on the stage, and focus on the specimen.

As shown in Fig. 11, adjust the interpupillary distance, so that both the right and left viewfields become one.

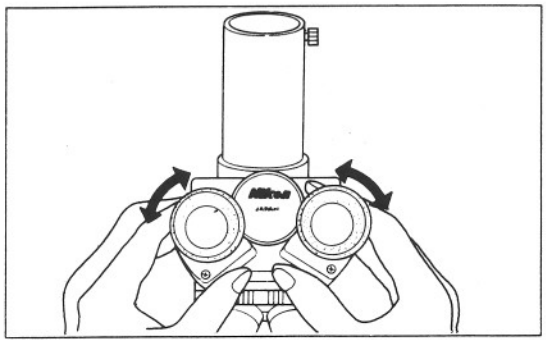


Fig. 11

3) Diopter adjustment

Make diopter adjustment for both the right and lefthand eyepieces.

(1) Turn the diopter ring on each eyepiece, until the end surface of the milled ring coincides with the engraved line, as shown in Fig. 12.

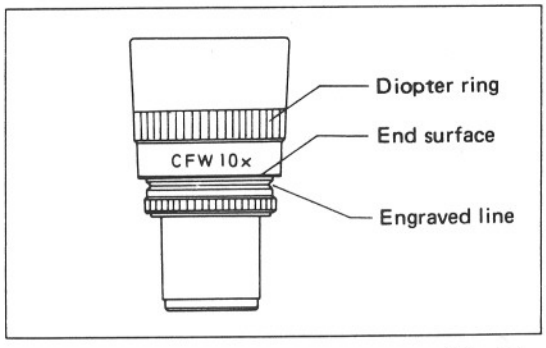


Fig. 12

(2) Mount the specimen on the stage. Swing the objective 40× into position, and bring the specimen image into focus. For facilitating the focusing, first use the 10× and then 40× objective.

(3) Thereupon, swing the objective 4× into position.
Without manipulating the coarse-and-fine focus knob, turn the diopter rings on the eyepieces, so that the specimen images in the right and lefthand eyepieces are focused individually.

- Repeat the above procedure two times, and a perfect diopter adjustment will be achieved.
- The above adjustment, compensating the diopter difference between the user's right and left eyes, will keep the tube length of microscope correct, thus enabling him to realize the full advantages of the high-class objectives, including their parfocality.

(4) Since the CF eyepieces are of high eyepoint type, it is not necessary for the user putting on his spectacles to remove them.
Only fold down the eyeguard rubber.
(Fig. 14)

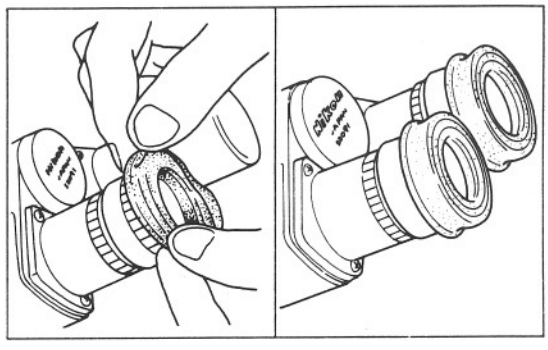


Fig. 13 Fig. 14

4) Optical path change-over in the trinocular eyepiece tube

(1) When using the trinocular eyepiece tube "F"

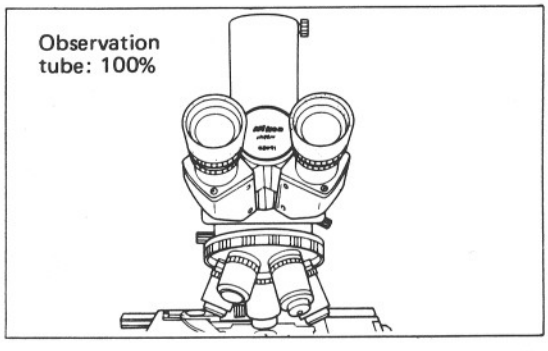


Fig. 15

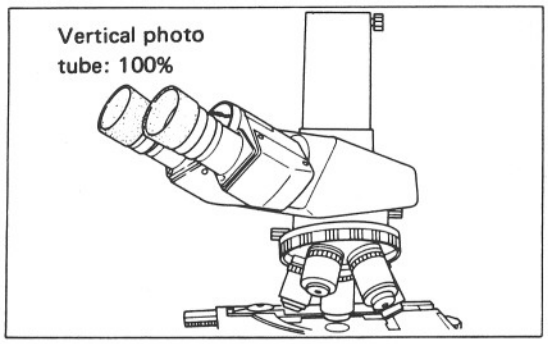


Fig. 16

As shown in Fig. 15, when the observation tube is turned toward the user, 100% of light enters the observation tube.

As shown in Fig. 16, when the observation tube is revolved 60° leftward, 100% of light enters the vertical photo tube.

In either case, turn the tube to the limit.

(2) When using the trinocular eyepiece tube "T" or the ultra wide eyepiece tube "UW"

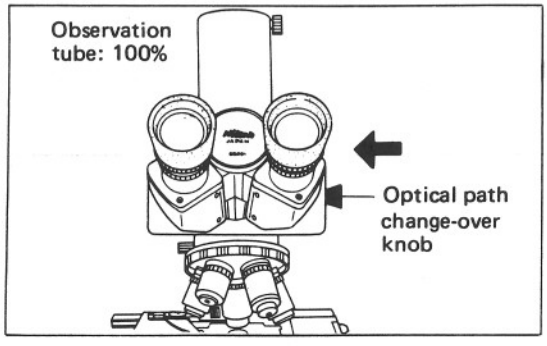


Fig. 17

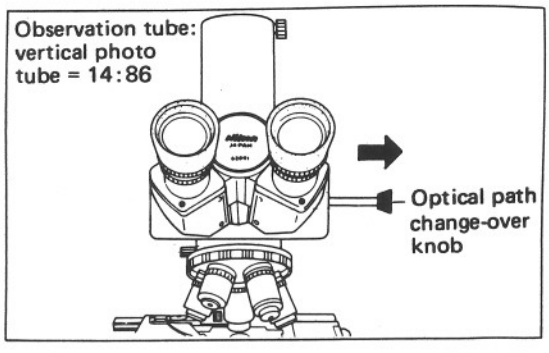


Fig. 18

As shown in Fig. 17, with the change-over knob pushed in, 100% of light enters the observation tube.

As shown in Fig. 18, with the change-over knob drawn out, the proportion of light entering the observation tube and photo tube will be 14 : 86.

5) 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 focus knob to move the condenser vertically so that a sharp image of the field diaphragm is formed on the specimen surface.

(2) Bring the field diaphragm image to the center of the field of view by means of the condenser centering screws. (Fig. 19- 1)

(3) Change over to the 40× 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. 19-2. If not centered, use the condenser centering screws again.

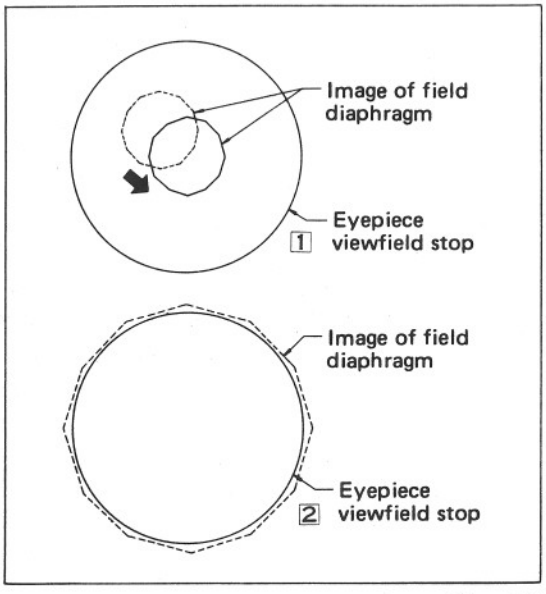


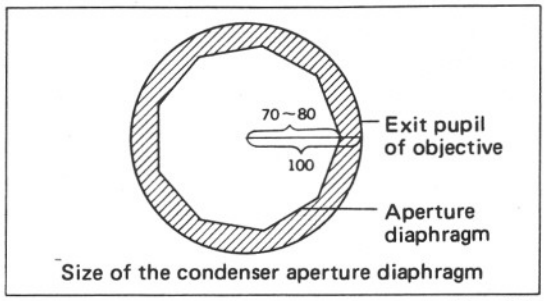
Fig. 19

6) Use 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. 20)



Size of the condenser aperture diaphragm

Fig. 20

To adjust the size of the condenser aperture diaphragm, turn the diaphragm control ring referring to the N.A. scale. Or, after removing the eyepiece from the eyepiece tube, adjust the size of the diaphragm, observing the image of the diaphragm which is visible on the bright circle of exit pupil of objective inside.

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.

7) Use 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. Generally, it is stopped down to such an extent that the circumference of the illuminated area circumscribes or inscribes that of the eyepiece field of view. 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.

8) 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. 21.

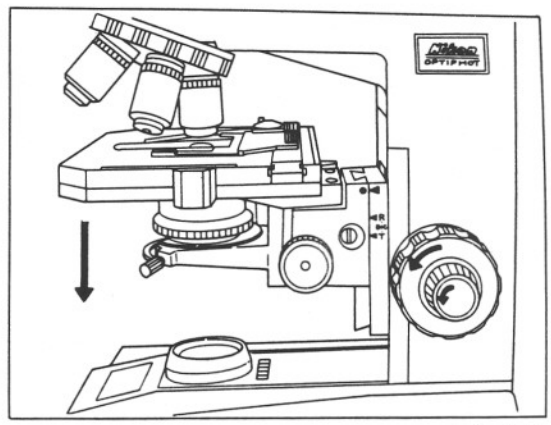


Fig. 21

One rotation of the fine focus knob moves the stage 0.1mm.

The graduation on this focus knob **is divided into 1μm.**

One rotation of the coarse focus knob moves the stage 4.7mm.

The range of coarse and fine motion is within 30mm; 2mm up and 28mm down from the standard position.

Tightness of the coarse-fine focus knob having been properly adjusted by the manufacturer, **it should never be readjusted in this model microscope by turning the one knob while holding the other.**

Don't rotate the coarse focus knob beyond the rotation limit.

9) Lowering the substage

Releasing the clamp screw using a screw driver, as shown in Fig. 22, permits lowering the substage as far as 32mm from the observing position beyond the moving stroke of the focusing device.

So, the microscope makes it possible to examine specimens as thick as 60mm, and is conveniently used for observing culture bottles and metallurgical specimens.

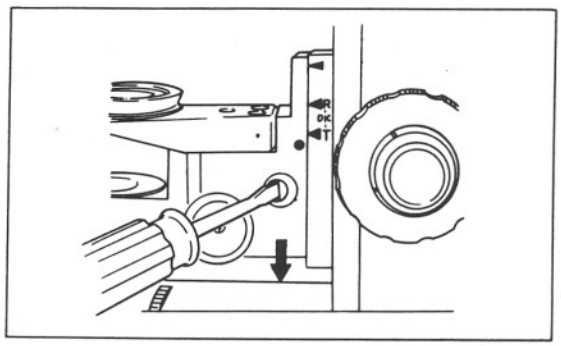


Fig. 22

V. OPTICAL SYSTEM

The CF objectives and CF eyepieces adopted in the Nikon Biological Microscope OPTIPHOT 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 Microscope OPTIPHOT 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 45mm (this is longer than the 33.6mm of earlier microscopes).

1) Types of objective

(1) Achromat (CF)

In this type of objective, the correction of chromatic aberrations is based on the lines C (red) and F (blue). Importance being given to the correction at the center of viewfield, the objectives offer the finest definition and highest contrast of image at the center. Even the 40× and 100× objectives fulfill the "Chromatic Aberration Free" correction, which has been considered difficult so far until now for such high magnifying powers. Furthermore, image flatness has been attained to an appreciable extent.

(2) Plan Achromat (CF Plan)

Same as the above type, the objectives accomplish the correction of chromatic aberrations based on the lines C and F.

In addition, owing to sufficient correction of all the image defects up to the periphery of viewfield, the objectives provide an unsurpassable high resolution and contrast of image over a wider field.

Focusing at the center means simultaneous focusing at the marginal part of viewfield. They are excellent for ultra-wide observation and photomicrography.

(3) Plan Apochromat (CF Plan Apo)

The use of fluorite and special, low color dispersion optical glasses improves the correction of chromatic aberrations over the entire visible region up to the line g (violet) along with the lines C and F.

These highest-grade objectives with their large numerical apertures produce an ideal image over a wide viewfield. With their outstanding definition, superior color reproducibility, and prominent image flatness, they are especially suited for most profound study of minute structures and color photomicrography.

(4) Epi-fluorescence (CF UV-F)

Exclusively designed for episcopic, fluorescence observation, this type objectives use non-fluorescent and non-solarisation materials and a strictly chosen cementing agent, to increase the transmission of UV exciting light (ultra-violet rays). Special weight being attached to the correction at the center of viewfield, and the numerical apertures made extremely large, they ensure bright and sharp fluorescence images using every excitation method. As immersion fluid, the objectives 10× ~ 100× of this type require the use of non-fluorescent glycerine of high purity.

2) Use of the objective

(1) Oil immersion objectives (Oil)

The objectives discriminated by the engraving "Oil" are to be **immersed in oil between the specimen and front of the objective.**

When using oil immersion objectives of numerical aperture 1.0 or higher, it is recommended, for making full use of its efficiency, to **use a high-class oil-immersion condenser such as of Achromat/aplanat type, applying oil between the glass slide and condenser as well.**

To see **if air bubbles are present in the immersion oil, which deteriorate the image quality,** pull out the eyepiece from the eye-

piece 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 engraved "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

The objective incorporating an iris diaphragm serves to cut off direct light in darkfield microscopy. Stop down the diaphragm nearly to its minimum opening.

2. Eyepieces

To take full advantage of the CF eyepieces, use them 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 with diopter ring are only used for observation. They are equipped with a rubber eyeguard.

An eyepiece called CFW 10×M, incorporating a photo mask, is also available, which enables focusing and framing by the use of the observation tube of the Trinocular Eyepiece Tube "T"

2) CFUW eyepiece (CFUW)

Featuring extra-wide field of view and high eyepoint, this eyepiece with diopter ring 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 tube.

An eyepiece called CFUW 10×M, incorporating a photo mask, is also available, which enables focusing and framing by the use of the observation tube of the Ultra Wide Eyepiece Tube "UW".

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 Achromat condenser

N.A. = 0.9. Dry system. It is used in combination with objectives from 2× to 100×, and provided with a swing-out top lens which is to be swung out when using the 2× or 4× objective. Its adjustable aperture scale is graduated in N.A. ratings.

2) Achromat/aplanat condenser

N.A. = 1.35. Oil system. The spherical, coma and chromatic aberrations being ideally corrected, this large aperture condenser is used with 10× ~ 100× 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 Apochromat objectives. When using the 100× objective for observation in combination with the CFW 10× eyepiece, it is possible to close the field diaphragm down to 45% of the viewfield.

4. Illumination System (Refer to Fig. 23)
The optical system for illumination in the OPTIPHOT microscope 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 50W (OSRAM 64610 or PHILIPS 7027). Four most commonly used filters are supplied in the base. (Refer to P. 11) Usually, place the diffuser in the optical path, but take away when the lamp is to be centered.

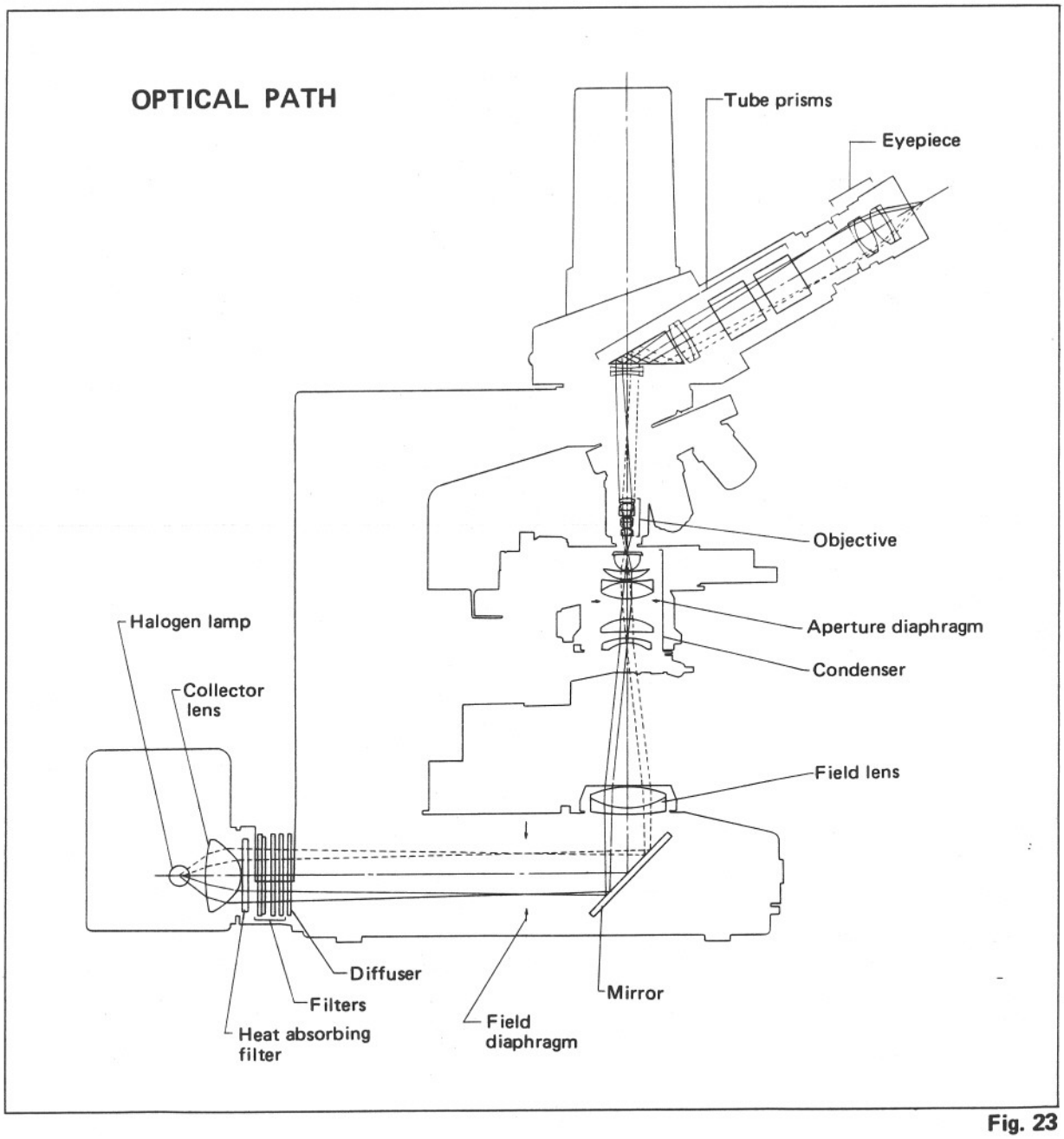


Fig. 23

5. Combination of Objectives and Eyepieces

Table 3.

CF Objectives (160/45) for Biological Microscope							CF Eyepieces											
							Ordinary viewfield									Ultra-Wide viewfield		
							CFW 8× Field number=18 (Now in preparation)			CFW 10× Field number=18			CFW 15× Field number=14			CFUW 10× Field number=26.5		
Type	Magnification	Numerical aperture N.A.	Working distance W.D. mm	Focusing distance f mm	Resolving power μm	Coverglass thickness mm	Total magnification M	Real viewfield φ mm	Depth of focus μm	Total magnification M	Real viewfield φ mm	Depth of focus μm	Total magnification M	Real viewfield φ mm	Depth of focus μm	Total magnification M	Real viewfield φ mm	Depth of focus μm
Plan Apochromat	2×	0.08	5.3	67.9	3.4	—	16×	9.0	232	20×	9.0	198	30×	7.0	154	20×	13.3	198
	4×	0.16	4.73	36.1	1.7	0.17	32×	4.5	58	40×	4.5	50	60×	3.5	39	40×	6.6	50
	10×	0.4	0.33	14.2	0.69	0.17	80×	1.8	9.3	100×	1.8	7.9	150×	1.4	6.2	100×	2.7	7.9
	With compensation ring 20×	0.65	0.5	7.9	0.42	0.17	160×	0.9	3.0	200×	0.9	2.6	300×	0.7	2.1	200×	1.3	2.6
	With compensation ring 40×	0.95	0.1	4.2	0.29	0.17 (0.11~0.23)	320×	0.45	1.2	400×	0.45	1.0	600×	0.35	0.8	400×	0.66	1.0
	60×	0.9	0.1	2.9	0.31	0.17 (0.11~0.23)	480×	0.3	1.0	600×	0.3	0.9	900×	0.23	0.8	600×	0.44	0.9
	Oil 100×	1.35	0.17	1.7	0.2	0.17	800×	0.18	0.4	1000×	0.18	0.4	1500×	0.14	0.3	1000×	0.27	0.4
	Oil NCG 100×	1.35	0.17	1.7	0.2	non	800×	0.18	0.4	1000×	0.18	0.4	1500×	0.14	0.3	1000×	0.27	0.4
Plan Achromat	1×	0.03	1.8	108.7	9.2	—	8×	18	1351	10×	18	1173	15×	14	935	—	—	—
	2×	0.05	5.8	70.1	5.5	—	16×	9.0	433	20×	9.0	379	30×	7.0	308	20×	13.3	379
	4×	0.1	13.8	40.4	2.8	—	32×	4.5	108	40×	4.5	95	60×	3.5	77	40×	6.6	95
	10×	0.25	7.1	16.7	1.1	—	80×	1.8	17	100×	1.8	15	150×	1.4	12	100×	2.7	15
	20×	0.4	1.4	8.4	0.69	0.17	160×	0.9	5.9	200×	0.9	5.3	300×	0.7	4.4	200×	1.3	5.3
	40×	0.65	0.48	4.1	0.42	0.17	320×	0.45	2.0	400×	0.45	1.8	600×	0.35	1.5	400×	0.66	1.8
	NCG 40×	0.65	0.45	4.2	0.42	non	320×	0.45	1.3	400×	0.45	1.2	600×	0.35	1.0	400×	0.66	1.2
	Oil 100×	1.25	0.2	1.8	0.22	0.17	800×	0.18	0.5	1000×	0.18	0.4	1500×	0.14	0.4	1000×	0.27	0.4
	With aperture diaphragm Oil 100×	1.25	0.2	1.8	0.22	0.17	800×	0.18	0.5	1000×	0.18	0.4	1500×	0.14	0.4	1000×	0.27	0.4
Achromat	4×	0.1	20	31.0	2.8	—	32×	4.5	108	40×	4.5	95	60×	3.5	77			
	10×	0.25	5.6	16.6	1.1	—	80×	1.8	17	100×	1.8	15	150×	1.4	12			
	20×	0.4	2.23	8.8	0.69	0.17	160×	0.9	5.9	200×	0.9	5.3	300×	0.7	4.4			
	40×	0.65	0.53	4.4	0.42	0.17	320×	0.45	2.0	400×	0.45	1.8	600×	0.35	1.5			
	Oil 100×	1.25	0.14	1.8	0.22	0.17	800×	0.18	0.5	1000×	0.18	0.4	1500×	0.14	0.4			
Epi-fluorescence UV-F	glycerin 10×	0.5	0.28	16.2	0.55	0.17	80×	1.8	6.9	100×	1.8	5.8	150×	1.4	4.4			
	glycerin 20×	0.8	0.2	8.9	0.34	0.17	160×	0.9	2.3	200×	0.9	2.0	300×	0.7	1.5			
	glycerin 40×	1.3	0.1	4.5	0.21	0.17	320×	0.45	0.8	400×	0.45	0.6	600×	0.35	0.5			
	glycerin 100×	1.3	0.13	1.7	0.21	0.17	800×	0.18	0.4	1000×	0.18	0.4	1500×	0.14	0.3			

Resolving power: $\frac{\lambda}{2\times N.A.}$

(λ = 0.55 μm standard wavelength)

Depth of focus:

$$\frac{n\times\lambda}{2\times(N.A.)^2}+\frac{n}{7\times N.A.\times M}$$

(Resolving power of eye = 2'
n: Refractive index of object side)

VI. PHOTOMICROGRAPHY

The Biological Microscope OPTIPHOT is 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. Selection of Voltage and Filter

The color temperature of the light source varies with the voltage being used. Therefore, in color photomicrography, the selection of voltage and filter is essential (for the result to be obtained).

1) Reading the brightness indicator

As shown in Fig. 24, take care to read the scale **vertically from above.**

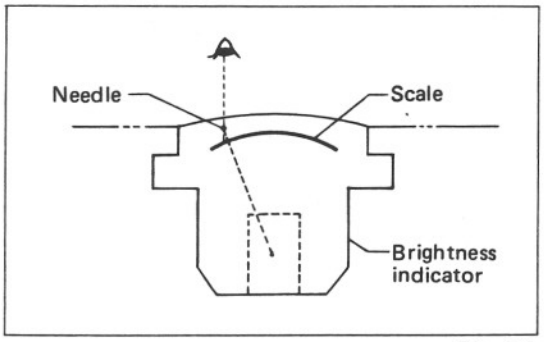


Fig. 24.

2) Selection of voltage and filter

Refer to the Table 4:

The NCB 10 filter is most suitable for a standard film. Depending upon the make of the film, different color renditions may result. It is recommended that in addition to the NCB 10 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. 27.

Table 4. Selection of Voltage and Filter

Film		Voltage	Filter
Color film	Daylight type	9	NCB 10 is to be used
	Tungsten type	8	Remove NCB 10
Monochrome film	———	Over 6	Remove NCB 10 Contrast filter(green), etc. is usable

4. Shutter Speed

Desirable shutter speeds for least vibration are **1/4 ~ 1/15 sec. Adjustment of the image brightness for color photomicrography should be made by means of the ND filters.**

5. 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.

6. Focusing

For focusing, refer to the Table 5:

Table 5. Focusing

	Focusing with 10× or higher objective	Focusing with 4× or lower objective
"F" tube	Use Microflex finder	Use Microflex finder + Focusing telescope
"T" or "UW" tube	Use observation tube	Use observation tube or Microflex finder + Focusing telescope

1) For focusing with the Microflex finder
Refer to the Instruction Manual for the Nikon Microflex.

2) Focusing with the observation tube
For focusing with the observation tube of eyepiece tube "T" or "UW" for photomicrography, use the eyepiece incorporating the photo mask.

Before proceeding to focusing, the binocular diopter adjustment should have been finished.

(1) Insert the eyepiece with photo mask into the eyepiece sleeve on the side of the user's dominant eye, and the viewing eyepiece into the other side sleeve.
Turning the diopter ring, bring the double crosshair in the mask eyepiece into sharp focus, and then, turning the coarse-fine focus knob, focus the specimen image onto the focused surface at the center of the mask. For diopter adjustment in the other eyepiece, do not manipulate the focus knob, but the diopter ring to bring the image into focus, with the objective 4× or 10×.

(2) Turning the eyepiece as a whole, set it in such a position that the photo mask appears as shown in Fig. 25.

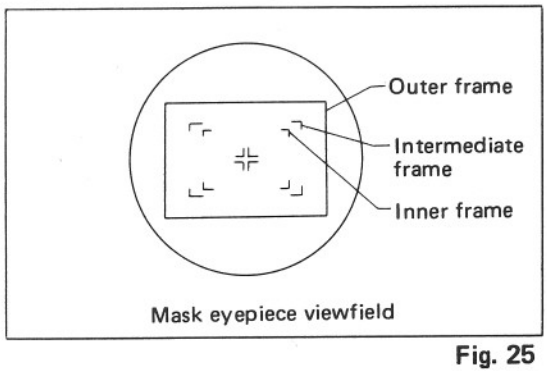


Fig. 25

(3) Furthermore, when using a low power objective, place the focusing telescope over the mask eyepiece, thus constructing an eyepiece of higher magnification, to perform precise focusing.

3) Magnifications of CF photo eyepieces suitable for each frame size of photo mask
Refer to Table 6.

Table 6. Magnifications of CF Photo Eyepieces Suitable for Frame Size of Photo Mask

	Type of film	Magnification of photographic lens	Magnification of photo eyepiece
Outer frame	35 mm	0.5×	5×
	3¼" × 4¼"	1.25×	
	6 × 9		
	4" × 5"		8×
Intermediate frame	35 mm	0.5×	8×
	3¼" × 4¼"	1.25×	
	6 × 9		
	4" × 5"		10×
Inner frame	35 mm	0.5×	10×
	3¼" × 4¼"	1.25×	
	6 × 9		

7. 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.

8. Others

- When using the 2× objective, it is recommended to remove the swing-out achromat condenser.
- When using the 1× objective, remove one of the ND filters and in place put the diffuser (available on order), and remove the condenser.
- For photomicrography, when focusing with the binocular observation tube, use the CF eyepiece, CF Photo eyepiece and CF Photo Mask eyepiece, with the magnification and other indications engraved in yellow, or in white with a white dot in addition.
- For the use of other photomicrographic attachments refer to the pertinent instruction manuals.

Ⅶ. USE OF THE ACCESSORIES

1. Ultra Wide Eyepiece Tube "UW"

1) Objectives

CF Plan Achromat 2X ~ 100X, CF Plan Apochromat 2X ~ 100X, CF Plan Achromat for phase contrast 10X ~ 100X, CF Plan Achromat for metallurgical 5X ~ 100X, CF Plan Apochromat for metallurgical 50X or CF BD Plan Achromat for bright and darkfield 5X ~ 100X are used.

2) Condenser

Refer to the Table 1 (P.10)

3) Assembly and microscopy

Assembly and microscopy being almost the same as that of the regular microscopy (P. 6 and P. 10), only the differences will be described below.

(1) Using the centering telescope

For attaching the centering telescope on top of the eyepiece sleeve, it is necessary to use the adapter (Fig. 26), because the telescope which has been originally designed for centering the annular diaphragm in phase contrast microscopy, has a fitting diameter different from that of the CFUW eyepiece.

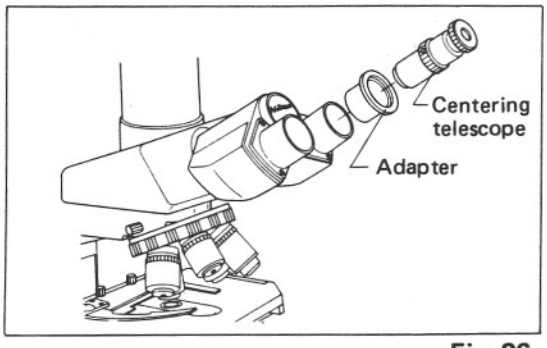


Fig. 26

2. Polarizing Filter Set "PT"

1) Nomenclature (Fig. 27)

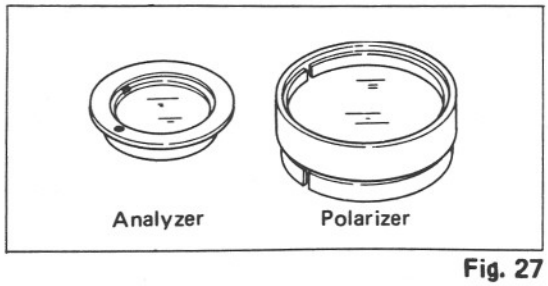


Fig. 27

2) Assembly

(1) Attaching the analyzer

After removing the eyepiece tube, insert the analyzer into the optical-path hole in the microscope arm. (Fig. 28)

The white index dot is to be brought into coincidence with the Y-axis (of X-Y coordinates), viewing the arm from above.

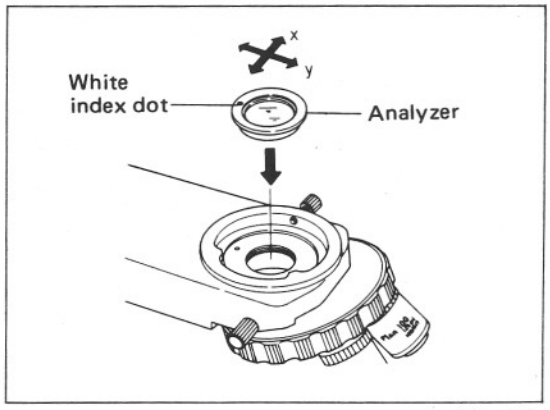


Fig. 28

(2) Condenser

Use the swing-out condenser.

(3) Attaching the polarizer

As shown in Fig. 29, fit the polarizer to the internal diameter at the bottom of the condenser.

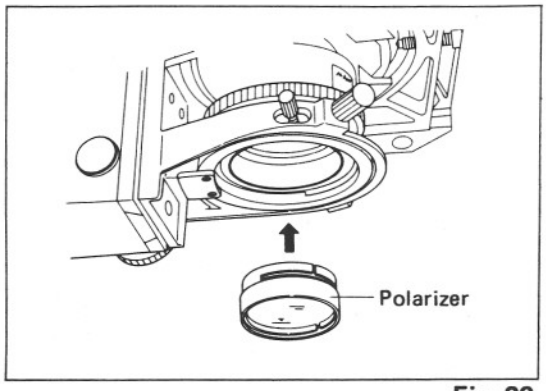


Fig. 29

(4) Objective

Use the ordinary CF objectives.

3) Microscopy

(1) Turn ON the power switch. Set the lamp voltage to 7 ~ 8 on the indicator.
(2) Remove the dust cap and put the NCB 10 filter.
(3) Place the specimen on the stage and focus on specimen with 10× objective.
(4) Adjust the interpupillary distance and diopter. (Refer to P. 11)
(5) Swing in the top lens of the swing-out condenser in the optical path. (If using 4× objective, swing out the top lens)
(6) Center the condenser. (Refer to P. 12)
(7) Rotate the polarizer until the darkest field of view is obtained.
(8) Adjust the brightness indicator to 10 ~ 11.
(9) Change over the objective to be used and sharpen the focus on the specimen.
(10) Adjust the aperture diaphragm and field diaphragm. (Refer to P. 12, 13)

Ⅷ. TROUBLE SHOOTING TABLE

Although nowhere the user can find any disorder or derangement in the instrument, if he encounters some difficulty or dissatisfaction, recheck the use, referring to the table below:

1. Optical

Failures	Causes ——→ Actions
Darkness at the periphery or uneven brightness of viewfield (No appearance of viewfield)	• Optical path in trinocular tube not fully changed-over ——→ Changing-over to the limit (Refer to P. 12) • Revolving nosepiece not in click-stop position (Objective not centered in optical path) ——→ Revolve it to click-stop position • Lamp bulb not centered ——→ Centering (Refer to P. 8) • Condenser not centered ——→ Centering by using field diaphragm (Refer to P. 12) • Field diaphragm too much closed ——→ Open it properly • Dirt or dust on the lens (Condenser, objective, eyepiece, slide) ——→ Cleaning • Improper use of condenser ——→ Correct use (Refer to P. 10) • Diffuser not set in or incorrectly positioned ——→ Correct positioning (Refer to P. 9) • Revolving nosepiece not correctly attached ——→ Correct attaching (Refer to P. 7)
Dirt or dust in the viewfield	• Dirt or dust on the lens (Condenser, objective, eyepiece, field lens) ——→ Cleaning • Dirt or dust on the slide ——→ Cleaning • Too low position of condenser ——→ Correct positioning (Refer to P. 12)
No good image obtained (low resolution or contrast)	• No coverglass attached to slide or NCG objective used with coverglass ——→ Correct use (Refer to P. 14) • Too thick or thin coverglass ——→ Use specified thickness (0.17mm) coverglass (Refer to P.15) • Immersion oil soils the top of dry system objective (especially 40×) ——→ Cleaning • Dirt or dust on the lens (condenser, objective, eyepiece, slide) ——→ Cleaning • No immersion oil used on immersion system objective ——→ Use immersion oil (Refer to P. 14) • Air bubbles in immersion oil ——→ Remove bubbles • Not specified immersion oil used ——→ Use Nikon immersion oil • Incorrect illumination ——→ Correct the illumination (Refer to P. 8) • Dirt or dust on the entrance lens ——→ Cleaning • Compensation ring in objective not adjusted ——→ Adjustment (Refer to P. 15) • Objective aperture (which provided) too much closed ——→ Open properly

Failures	Causes ——→	Actions
Image quality deteriorated	● Condenser aperture too much closed ——→	Open properly (Refer to P. 12)
	● Too low position of condenser ——→	Bring it up to coincidence with field diaphragm image (Refer to P. 12)
	● Diffuser not inserted ——→	Insert it in correct position (Refer to P. 9)
Oneside dimness of image	● Revolving nosepiece not in click-stop position ——→	Revolve it to click-stop position
	● Revolving nosepiece not correctly attached ——→	Insert it to the limit and clamp it firmly
	● Revolving nosepiece not clamped ——→	Clamp tightly
Image moves while being focused	● Specimen rises from stage surface ——→	Place it stable
	● Revolving nosepiece not in click-stop position ——→	Revolve it to click-stop position
	● Revolving nosepiece not clamped ——→	Clamp tightly
	● Condenser not correctly centered ——→	Correct centering (Refer to P.12)
	● Lamp bulb not correctly centered ——→	Correct centering (Refer to P.8)
Image tinged yellow	● NCB 10 filter not used ——→	Use NCB 10 filter
	● Too low power source voltage ——→	Raise the voltage over 6 on the indicator
Too bright image	● ND filter not used ——→	Use ND filter

2. Manipulation

Failures	Causes ——→	Actions
No focused image obtained with high power objectives	● Upside down of slide ——→	Turn over the slide
	● Too thick coverglass ——→	Use specified thickness (0.17mm) coverglass (Refer to P. 15)
High power objective touches the slide, when changed-over from low power	● Upside down of slide ——→	Turn over the slide
	● Too thick coverglass ——→	Use specified thickness (0.17mm) coverglass (Refer to P. 15)
	● Eyepiece diopter not adjusted (Especially when changing-over low power objective 1× or 2×) ——→	Diopter adjustment (Refer to P. 11)
Insufficient parfocality of objective(when changed-over)	● Eyepiece diopter not adjusted ——→	Diopter adjustment (Refer to P. 11)
Movement of image not smooth by moving the slide	● Slide holder not tightly fixed ——→	Fix it tightly

Failures	Causes ——→	Actions
Travel of stage limited to one half length of slide	• Improper attaching of slide holder ——→	Shift the attaching position
No fusion of binocular images	• Interpupillary distance not adjusted ——→	Adjustment (Refer to P. 11)
Fatigue of observing eyes	• Incorrect diopter adjustment ——→	Correct adjustment (Refer to P. 11)
	• Inadequate brightness of illumination ——→	Use ND filter or change power voltage

3. Electrical

Failures	Causes ——→	Actions
Lamp does not light even though switched ON	• No electricity obtained ——→	Connect the cord to socket
	• No lamp bulb attached ——→	Attaching
	• Lamp bulb blown ——→	Replacement
	• Fuse blown ——→	Replacement
Unstable brightness of illumination	• Input voltage not adjusted to house current voltage ——→	Turn the change-over switch on the microscope bottom
	• House current voltage fluctuates too much ——→	Use transformer or the like (for adequate voltage)
	• Lowest voltage adjustment not made ——→	Make adjustment (Refer to P. 9)
Strong glare even at lowest voltage, when using low power objective	• Lowest voltage adjustment not made ——→	Make adjustment (Refer to P. 9)
Lamp bulb promptly blown	• Not specified lamp bulb used ——→	Use 12V 50W specified lamp bulb: (Halogen bulb: OSRAM 64610 or PHILIPS 7027)
	• Too high voltage of house current ——→	Use transformer for adjustment
Insufficient brightness of illumination	• Lamp bulb not centered ——→	Centering (Refer to P. 8)
	• Condenser not centered ——→	Centering (Refer to P. 12)
	• Condenser aperture too much closed ——→	Open it properly (Refer to P. 12)
	• Too low position of condenser ——→	Correct positioning (Refer to P. 12)
	• Not specified lamp bulb used ——→	Use 12V 50W specified Halogen bulb
	• Dirt on lens (condenser, objective, eyepiece, field lens, filter) ——→	Cleaning
	• Too low voltage ——→	Raise the voltage
Fuse blown	• Not specified fuse used ——→	Use 1A (250V) or 0.75A (250V)

Failures	Causes ⟶ Actions
Flickering or unstable brightness of lamp bulb	• Lamp bulb going to be blown ⟶ Replacement • Connector not connected securely ⟶ Secure connection • Fuse holder not firmly fastened ⟶ Firm fastening • Irregular change of house current voltage ⟶ Use stabilizer • Lamp bulb insufficiently inserted into the socket ⟶ Positive connection

4. Photomicrography

Failures	Causes ⟶	Actions
No sharp picture obtained	• Improper focusing ⟶	• Viewing into the finder and turning diopter ring, bring double crosshair into focus. Moving the eye laterally, rotate fine focus knob, until no parallax separation appears between the image and double crosshair. • At lower magnifications use focusing telescope in addition.
	• Out of focus (Especially with high power objective and long-exposure) ⟶	• For preventing external vibration, use vibration-proof table or rigid desk. • Select a place free from vibrations, such as caused by traffic, passers-by or motors etc.
	• Momentary vibration ⟶	• Using ND filters or others, elongate exposure time (for color film, to 1/4 ~ 1/15 sec.) • Lower the voltage, and elongate exposure time (for (black-and-white film). Note, however, for color film, that lowering of color temperature and change of spectral characteristics will be unavoidable. • Use photomicrographic stand and separate camera from microscope to prevent transmission of vibration.
	• Incorrect thickness of coverglass (Especially, when using large N.A. and high power objective ⟶	• Use a standard coverglass of 0.17mm in thickness (No. 1 ½). • Use objective with coverglass thickness compensation ring.
	• Using dry objectives for smear preparations ⟶	• Use no-coverglass type objective. • If other objectives are to be used, place a coverglass on the specimen.
Fogging of image	• Grease, dust or dirt on optical surfaces ⟶	• Clean the front of objective thoroughly, top surface of photo eyepiece, specimen, photographic lens, condenser lens, field lens, etc.
Illuminated image not uniformly	• Inadequate adjustment of illumination (This shows up more conspicuously in photography than in observation) ⟶	• Adjust illumination properly (Refer to P. 8).
Insufficient image contrast	• Aperture diaphragm opened too large. ⟶	• Generally, good results will be achieved with aperture stopped down to 70~80% of N.A. of the objective being used. (Refer to P.13).

Failures	Causes ——→	Actions
	• Incorrect use of filter ——→	• In metallurgical, interference, polarizing or phase contrast microscopy, use of a green filter or monochromatic interference filter (e.g. peak wavelength = 546nm, half-value range = 30nm) will increase contrast. • When contrast is to be increased for a part stained with a particular color, use a filter whose color is complementary to the stain color (for black-and-white film).
	• Inadequate use of field diaphragm ——→	• Stop down field diaphragm to a diameter slightly larger than the diagonal of picture frame. (Refer to P. 18)
	• Low contrast in specimen ——→	• To increase contrast optically, select phase contrast, darkfield, or differential interference methods. • Specimens should be stained a rather dark color, if possible. • In color photography, depending upon the specimen, red-blue separation staining (Mallory or Azan methods etc.) is preferable to red-violet combination staining (H-E staining). • In black-and-white photography, for low contrast specimens a film of finer grain and higher contrast is more suited (such as minicopy film). • For general specimens a film of wider latitude and finer grain is preferable.
Deficient resolving power of microscope	• Insufficient N.A. of objective ——→	• Use a large N.A. objective. • For the same magnification, increase power of objective rather than that of eyepiece to attain higher resolution and sharpness, even though depth of field is reduced.
	• Excessive magnification ——→	• 500~1000 times N.A. are magnification limits for resolving power.
Ghosts or flare appears	• Extraneous light entering the ocular finder ——→	• Darken the surroundings or place the cap on the ocular finder.
	• Stray light entering ——→	• Take care not to expose microscope and specimen to direct sunlight and other intense lights.
Poor photograph obtained	• Inadequate use of filter ——→	• Select best filter combination. Refer to Table, "Combination of Film and Filter" P.27.
	• Film of another make or emulsion NO. ——→	• Note that, when using a daylight film, remarkably different spectral sensitivities will result depending upon the type, make, etc. • Even though of same make, according to emulsion number, different color rendition will be obtained.
	• Wrong power source voltage used ——→	• Take picture in every case at the specified voltage. (Refer to P.18 Table 4)
	• Incorrect exposure time ——→	• By inadequate exposure time, color rendition will not be true on account of "reciprocity law failure" Then, with the help of exposure time indicator, adjust exposure time according to characteristics of film by means of ND filters, or compensate for such failure by means of CC filters. (Refer to Kodak Data)
	• Influenced by film development ——→	• Especially, for making color prints, it is recommended to contact the development laboratory.

SOME DATA ON COLOR PHOTOMICROGRAPHY USING OPTIPHOT MICROSCOPE

We have designed the OPTIPHOT microscope 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)	Accessory filter (NCB 10)	Lamp voltage (Indicator)	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 64 DAYLIGHT (64)	Used	9	Not used	64	1/15* (1/4~1/30) **	Good balancing of colors and easily usable
Agfa CT 18 (50)	Used	11	Not used	50	1/15* (1/4~1/30) **	Good resolution, color balance and background tone
Fujichrome 100RD (100)	Used	9	Not used	100	1/15* (1/2~1/30) **	Good balancing of colors and easily usable
Sakurachrome 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 supplied as accessories.

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

ELECTRICAL SPECIFICATIONS

Power source	100/120V 220/240V 50/60 Hz
Halogen lamp	12V 50W (OSRAM 64610 or PHILIPS 7027)
Fuse	100/120V 1A (250V) 220/240V 0.75A (250V)

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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